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UNITED STATES DISTRICT COURT
DISTRICT OF NEW JERSEY

MERCK SHARP & DOHME CORP.,
CUBIST PHARMACEUTICALS LLC,
OPTIMER PHARMACEUTICALS LLC,
MSD INVESTMENT HOLDINGS (IRELAND), and
MSD INTERNATIONAL GMBH,

Plaintiffs,

v.

ACTAVIS LABORATORIES FL, INC.,
ACTAVIS PHARMA, INC., and
TEVA PHARMACEUTICALS USA, INC.,

Defendants.

Civil Action No. _____

(Filed Electronically)

COMPLAINT FOR PATENT INFRINGEMENT

Plaintiffs Merck Sharp & Dohme Corp. ("Merck"), Cubist Pharmaceuticals LLC ("Cubist"), Optimer Pharmaceuticals LLC ("Optimer"), MSD Investment Holdings (Ireland) ("MSD Investment Ireland"), and MSD International GmbH ("MSD International") (collectively, "Plaintiffs") for their Complaint against Defendants Actavis Laboratories FL, Inc., Actavis Pharma, Inc., and Teva Pharmaceuticals USA, Inc. (collectively, "Actavis " or "Defendants"), hereby allege as follows:

NATURE OF THE ACTION

1. This is an action for patent infringement arising under the patent laws of the United States, Title 35, United States Code, § 100 *et seq*. This action relates to Abbreviated New Drug Application ("ANDA") No. 208443, and amendments made to the ANDA ("Amended ANDA" or "Actavis's Amended ANDA"), which Defendants filed or caused to be filed with the U.S. Food and Drug Administration ("FDA") seeking approval to engage in the commercial manufacture, use, sale, offer for sale, and/or importation of generic copies of Plaintiffs' DIFICID® (fidaxomicin) Tablets prior to the expiration of U.S. Patent No. 7,906,489 ("the '489 Patent"), U.S. Patent No. 8,586,551 ("the '551 Patent"), U.S. Patent No. 7,378,508 ("the '508 Patent"), U.S. Patent No. 7,863,249 ("the '249 Patent"), and U.S. Patent No. 8,859,510 ("the '510 Patent").

2. Plaintiffs previously filed Case No. 2:15-cv-06541-CCC-CLW in this District in response to the original submission of ANDA No. 208443 to the FDA. Plaintiffs now file the instant action in response to Defendants' submission to the FDA of Amended ANDA No. 208443. This action and Case No. 2:15-cv-06541-CCC-CLW both involve the validity and infringement of the same patents.

PARTIES

3. Plaintiff Merck is a corporation organized and existing under the laws of the State of New Jersey, having a principal place of business at 1 Merck Drive, Whitehouse Station, New Jersey, 08889. Merck is a global research-driven pharmaceutical company that discovers, develops, manufactures and markets a broad range of innovative products to improve health.

4. Plaintiff Cubist is a limited liability company organized and existing under the laws of the State of Delaware, having a principal place of business at 2000 Galloping Hill Road, Kenilworth, New Jersey 07033. Cubist is a wholly owned subsidiary of Merck & Co., Inc. Plaintiff Cubist was formerly known as Cubist Pharmaceuticals, Inc.

5. Plaintiff Optimer is a limited liability company organized and existing under the laws of the State of Delaware, having a principal place of business at 2000 Galloping Hill Road, Kenilworth, New Jersey 07033. Optimer is a wholly owned subsidiary of Merck & Co., Inc. Plaintiff Optimer was formerly known as Optimer Pharmaceuticals, Inc.

6. Plaintiff MSD Investment Ireland is an unlimited liability company incorporated under the laws of Ireland with registered number 463181 and having its registered office at Ballydine, Kilsheelan, Clonmel, Co Tipperary, Ireland and its principal place of business at Weystrasse 20, 6000 Lucerne 6, Switzerland. MSD Investment Ireland is a wholly owned subsidiary of Merck & Co., Inc.

7. Plaintiff MSD International is a Swiss limited liability company having its registered office at Weystrasse 20, 6000 Lucerne 6, Switzerland. MSD International is a wholly owned subsidiary of Merck & Co., Inc.

8. Defendant Actavis Laboratories FL, Inc. ("Actavis Labs. FL") is a corporation organized and existing under the laws of the State of Florida, having a principal place of business at 4955 Orange Drive, Davie, Florida 33314, and having a President, Treasurer, and Director

who each reside in Parsippany, NJ. Actavis Labs. FL is an indirect wholly-owned subsidiary of Teva Pharmaceuticals USA, Inc., which itself is an indirect wholly-owned subsidiary of Teva Pharmaceuticals Industries Ltd. On information and belief, Actavis Labs. FL is in the business of, among other things, developing and manufacturing generic pharmaceutical products and obtaining regulatory approval for generic pharmaceutical products that it distributes in New Jersey and throughout the United States.

9. Defendant Actavis Pharma, Inc. ("Actavis Pharma") is a corporation organized and existing under the laws of the State of Delaware, having a principal place of business at Morris Corporate Center III, 400 Interpace Parkway, Parsippany, New Jersey 07054. Actavis Pharma is a wholly-owned subsidiary of Teva Pharmaceuticals Industries Ltd. Actavis Pharma is registered with the State of New Jersey's Division of Revenue and Enterprise Services as a business operating in New Jersey under Business ID No. 0100573928 and has appointed Corporate Creations Network Inc., 811 Church Road #105, Cherry Hill, New Jersey 08002, as its registered agent for service of process in New Jersey. On information and belief, Actavis Pharma is in the business of, among other things, distributing and/or selling generic pharmaceutical products, including those that are manufactured by Actavis Labs. FL, in New Jersey and throughout the United States.

10. Defendant Teva Pharmaceuticals USA, Inc. ("Teva USA") is a corporation organized and existing under the laws of the State of Delaware, having a principal place of business at 1090 Horsham Road, North Wales, PA 19454, and having a Senior Vice President who resides in Parsippany, New Jersey and a place of business at 400 Interpace Parkway, Parsippany, NJ 07054. Teva USA is a wholly-owned subsidiary of Teva Pharmaceuticals Industries Ltd. Teva USA is registered with the State of New Jersey's Division of Revenue and

Enterprise Services as a business operating in New Jersey under Business ID No. 0100250184 and has appointed Corporate Creations Network Inc., 811 Church Road #105, Cherry Hill, New Jersey 08002, as its registered agent for service of process in New Jersey. On information and belief, Teva USA, at least through the actions of its subsidiaries, including Actavis Labs. FL, is in the business of, among other things, developing, manufacturing, obtaining regulatory approval for, marketing, distributing, and selling generic pharmaceutical products, including those that are manufactured by Actavis Labs. FL, in New Jersey and throughout the United States.

JURISDICTION AND VENUE

11. This action for patent infringement arises under the patent laws of the United States of America, 35 U.S.C. § 100 *et seq.*

12. This Court has subject-matter jurisdiction over this dispute pursuant to 28 U.S.C. §§ 1331, 1338(a), 2201, and 2202.

13. Venue is proper in this Judicial District pursuant to 28 U.S.C. §§ 1391 and 1400(b).

14. This Court has personal jurisdiction over Defendants by virtue of their specific acts in, and their continuous and systematic contacts with, the State of New Jersey.

15. This Court has personal jurisdiction over Actavis Labs. FL by virtue of, among other things: (1) its continuous and systematic contacts with New Jersey; (2) its acts of patent infringement that will result in foreseeable harm to Plaintiffs in New Jersey; (3) its purposefully availing itself of the jurisdiction of this Court in the past; and (4) its conduct by, through, and in concert with Actavis Pharma and Teva USA.

16. This Court has personal jurisdiction over Actavis Pharma by virtue of, among other things: (1) its continuous and systematic contacts with New Jersey, including its principal

place of business in Parsippany, New Jersey; (2) its acts of patent infringement that will result in foreseeable harm to Plaintiffs in New Jersey; (3) its sale of a substantial volume of pharmaceutical products in New Jersey; (4) its purposefully availing itself of the jurisdiction of this Court in the past; (5) its consent to jurisdiction in New Jersey by its registration to do business in New Jersey and appointment of a registered agent in New Jersey for the receipt of service of process; and (6) its conduct by, through, and in concert with Actavis Labs. FL and Teva USA.

17. This Court has personal jurisdiction over Teva USA by virtue of, among other things: (1) its continuous and systematic contacts with New Jersey, including its place of business in Parsippany, New Jersey; (2) its acts of patent infringement that will result in foreseeable harm to Plaintiffs in New Jersey; (3) its sale of a substantial volume of pharmaceutical products in New Jersey; (4) its purposefully availing itself of the jurisdiction of this Court in the past; (5) its consent to jurisdiction in New Jersey by its registration to do business in New Jersey and appointment of a registered agent in New Jersey for the receipt of service of process; and (6) its conduct by, through, and in concert with Actavis Labs. FL and Actavis Pharma.

18. As noted above, on information and belief, Actavis Labs. FL has substantial, continuous and systematic contacts with New Jersey.

19. Further, Actavis Labs. FL has committed an act of patent infringement under 35 U.S.C. § 271(e)(2), and intends a future course of conduct that includes acts of patent infringement in New Jersey. These acts have led and will lead to foreseeable harm and injury to Plaintiffs, including harm and injury in New Jersey. For example, on information and belief, Actavis Labs. FL is actively preparing to make generic copies of DIFICID® (fidaxomicin)

Tablets that are the subject of Actavis's Amended ANDA No. 208443, and is preparing to commercially manufacture, use, sell, offer to sell, and/or import such generic copies in this State and this Judicial District immediately upon approval of Actavis's Amended ANDA.

20. Actavis Labs. FL has previously submitted to the jurisdiction of this Court and asserted counterclaims in this jurisdiction, including in related litigation concerning the original ANDA No. 208443 and the same patents that are at issue in this Complaint. *See Merck, Sharp & Dohme Corp., et al. v. Actavis Labs. FL, Inc., et al.*, Civil Action No. 15-6541; *see also, e.g.*, *Bausch Health Companies Inc., et al. v. Actavis Labs. FL, Inc., et al.*, Civil Action No. 19-13722; *Sebela Int'l Ltd., et al. v. Actavis Labs. FL, Inc.*, Civil Action No. 17-4789; *Impax Labs., Inc. v. Actavis Labs. FL, Inc., et al.*, Civil Action No. 17-3295; *Valeant Pharms. Int'l, Inc., et al. v. Actavis Labs. FL Inc., et al.*, Civil Action No. 17-12857; *Horizon Pharma, Inc., et al. v. Actavis Labs. FL, Inc., et al.*, Civil Action No. 15-3322; *Supernus Pharms., Inc. v. Actavis Inc., et al.*, Civil Action No. 15-2499; *Vivus, Inc. v. Actavis Labs. FL, Inc., et al.*, Civil Action No. 15-1636; *Orexo Ab v. Actavis Labs. FL, Inc., et al.*, Civil Action No. 15-826; *Astrazeneca Ab, et al. v. Actavis Labs. FL, Inc., et al.*, Civil Action No. 14-7870; *Astrazeneca Ab, et al. v. Actavis Labs. FL, Inc., et al.*, Civil Action No. 14-7263; *Noven Therapeutics, LLC v. Actavis Labs. FL, Inc., et al.*, Civil Action No. 14-6414; *Supernus Pharms., Inc. v. Actavis Inc., et al.*, Civil Action No. 14-6102; and *Vivus, Inc. v. Actavis Labs. FL, Inc., et al.*, Civil Action No. 14-3786.

21. As noted above, on information and belief, Actavis Pharma has substantial, continuous and systematic contacts with New Jersey, including, *inter alia*, having a principal place of business in New Jersey and an appointed registered agent for service of process in New Jersey.

22. Further, Actavis Pharma, at least through the actions of its affiliates Actavis Labs. FL and Teva USA, has committed an act of patent infringement under 35 U.S.C. § 271(e)(2), and intends a future course of conduct that includes acts of patent infringement in New Jersey. These acts have led and will lead to foreseeable harm and injury to Plaintiffs, including harm and injury in New Jersey. For example, on information and belief, Actavis Pharma is actively preparing to commercially manufacture, use, sell, offer to sell, and/or import generic copies of DIFICID® (fidaxomicin) Tablets that are the subject of Actavis's Amended ANDA No. 208443, and is preparing to commercially manufacture, use, sell, offer to sell, and/or import such generic copies in this State and this Judicial District immediately upon approval of Actavis's Amended ANDA.

23. Actavis Pharma has also previously submitted to the jurisdiction of this Court and asserted counterclaims in this jurisdiction, including in related litigation concerning the original ANDA No. 208443 and the same patents that are at issue here. *See Merck, Sharp & Dohme Corp., et al. v. Actavis Labs. FL, Inc., et al.*, Civil Action No. 15-6541; *see also*, *e.g.*, *Sebela Int'l Ltd., et al. v. Actavis Labs. FL, Inc.*, Civil Action No. 17-4789; *Impax Labs., Inc. v. Actavis Labs. FL, Inc., et al.*, Civil Action No. 17-3295; *Horizon Pharma, Inc., et al. v. Actavis Labs. FL, Inc., et al.*, Civil Action No. 15-3322; *Supernus Pharms., Inc. v. Actavis Inc., et al.*, Civil Action No. 15-2499; *Astrazeneca Ab, et al. v. Actavis Labs. FL, Inc.*, *et al.*, Civil Action No. 14-7870; *Astrazeneca Ab, et al. v. Actavis Labs. FL, Inc., et al.*, Civil Action No. 14-7263; *Noven Therapeutics, LLC v. Actavis Labs. FL, Inc., et al.*, Civil Action No. 14-6414; *Supernus Pharms., Inc. v. Actavis Inc., et al.*, Civil Action No. 14-6102; *Supernus Pharms., Inc. v. Actavis Inc., et al.*, Civil Action No. 14-1981; and *Bayer Pharma AG, et al. v. Watson Labs., Inc., et al.*, Civil Action No. 14-1804.

24. As noted above, on information and belief, Teva USA also has substantial, continuous and systematic contacts with New Jersey, including *inter alia*, having a registration to do business in New Jersey and an appointed registered agent for service of process in New Jersey.

25. Further, Teva USA, at least through the actions of its affiliates Actavis Labs. FL, and Actavis Pharma, has committed an act of patent infringement under 35 U.S.C. § 271(e)(2), and intends a future course of conduct that includes acts of patent infringement in New Jersey. These acts have led and will lead to foreseeable harm and injury to Plaintiffs, including harm and injury in New Jersey. For example, on information and belief, Teva USA is actively preparing to commercially manufacture, use, sell, offer to sell, and/or import generic copies of DIFICID® (fidaxomicin) Tablets that are the subject of Actavis's Amended ANDA No. 208443, and is preparing to commercially manufacture, use, sell, offer to sell, and/or import such generic copies in this State and this Judicial District immediately upon approval of Actavis's Amended ANDA.

26. Teva USA too has previously submitted to the jurisdiction of this Court and asserted counterclaims in this jurisdiction. *See, e.g.*, *Inspiron Delivery Sciences, LLC v. Teva Pharm. USA, Inc., et al.*, Civil Action No. 19-10464; *Celgene Corp. v. Teva Pharm. USA, Inc., et al.*, Civil Action No. 19-8758; *Celgene Corp. v. Teva Pharm. USA, Inc., et al.*, Civil Action No. 18-14366; *Celgene Corp. v. Teva Pharm. USA, Inc., et al.*, Civil Action No. 18-11215; *Gilead Sciences, Inc., et al. v. Teva Pharm. USA, Inc.*, Civil Action No. 18-4273; *Adapt Pharma Operations Ltd., et al. v. Teva Pharm. USA, Inc., et al.*, Civil Action No. 18-9880; *Janssen Pharm., Inc., et al. v. Teva Pharm. USA, Inc., et al.*, Civil Action No. 18-734.

27. On information and belief, Actavis Labs. FL, Actavis Pharma, and Teva USA hold themselves out as a single entity for the purposes of manufacturing, selling, marketing,

distributing, and importing generic drug products in New Jersey and throughout the United States.

28. More specifically, Defendants, on information and belief, collectively share common directors, officers, principals and/or facilities, operate as agents of each other and act in concert with each other in the design, formulation, development, manufacture, packaging, distribution, marketing and/or sale of pharmaceutical products throughout the United States, including New Jersey, and will do the same with respect to Actavis's product for which they have sought approval from the FDA in Amended ANDA No. 208443.

29. On information and belief, Actavis Labs. FL, Actavis Pharma, and Teva USA operate as an integrated business ultimately owned and controlled by Teva Pharmaceuticals Industries Ltd.

30. On information and belief, Defendants have sold a substantial volume of generic pharmaceutical products in New Jersey.

31. On information and belief, Defendants conduct marketing and sales activities in the State of New Jersey, including, but not limited to, the systemic and continuous distribution, marketing and sales of generic pharmaceutical products to New Jersey residents.

32. On information and belief, Defendants acted in concert to develop a generic copy of DIFICID® (fidaxomicin) Tablets and to seek approval from the FDA to sell generic copies of DIFICID® (fidaxomicin) Tablets in New Jersey and throughout the United States.

33. On information and belief, Actavis Pharma and Teva USA, together with and/or through their affiliate and/or agent, Actavis Labs. FL, filed the Amended ANDA No. 208443, which is at issue in this patent-infringement suit, with the FDA.

34. Plaintiffs' claim for patent infringement arose as a result of Actavis Labs. FL sending the required notice of its Amended ANDA. The notice was sent on behalf of Actavis Labs. FL by Teva USA and stated that Actavis Labs. FL is an indirect, wholly owned subsidiary of Teva USA. The notice identified the contact information for Teva USA's in-house counsel as Teva Pharmaceuticals USA, Inc., 400 Interpace Parkway, Parsippany, NJ 07054.

35. On information and belief, Actavis Pharma and Teva USA, together with their affiliate and/or agent, Actavis Labs. FL, have committed, or aided, abetted, actively induced, contributed to, or participated in the commission of an act of patent infringement under 35 U.S.C. § 271(e)(2) that has led and/or will lead to foreseeable harm and injury to Plaintiffs, including harm and injury in New Jersey.

PLAINTIFFS' DIFICID® (FIDAXOMICIN) TABLETS

36. Plaintiff Cubist is the holder of New Drug Application ("NDA") No. 201699 that has been approved by the FDA for the manufacture and sale of DIFICID® (fidaxomicin) Tablets for oral use ("DIFICID®" or the "DIFICID® drug product"). Plaintiff Cubist was formerly known as Cubist Pharmaceuticals, Inc. On October 24, 2013, Cubist Pharmaceuticals, Inc. acquired Optimer Pharmaceuticals, Inc., which is the entity that originally filed NDA No. 201699. Optimer Pharmaceuticals, Inc. subsequently became Optimer Pharmaceuticals LLC.

37. DIFICID® is approved by the FDA for the treatment of *Clostridium difficile*-associated diarrhea in adults 18 years of age or older. Under NDA No. 201699, DIFICID® is marketed in 200 mg tablets. The drug is marketed under the registered trade name and trademark DIFICID®.

THE PATENTS-IN-SUIT

The '489 Patent

38. The '489 Patent, entitled "18-Membered Macrocycles and Analogs Thereof," was duly and legally issued by the United States Patent and Trademark Office ("USPTO") on March 15, 2011, naming Youe-Kong Shue, Chan-Kou Hwang, Yu-Hung Chiu, Alex Romero, Farah Babakhani, Pamela Sears, and Franklin Okumu as the inventors. A copy of the '489 Patent is attached hereto as Exhibit 1.

39. Plaintiff Merck is the owner, by assignment, of the '489 Patent and has the full right to sue and to recover for infringement thereof. Plaintiff Optimer previously owned the '489 Patent and retains certain interests in the '489 Patent. And Plaintiffs MSD Investment Ireland and MSD International have certain rights in the '489 Patent by license.

40. The '489 Patent is listed in the FDA's publication, *Approved Drug Products with Therapeutic Equivalence Evaluations* (the "Orange Book") as covering the drug DIFICID®, at the dosage of 200 mg, which is the subject of approved NDA No. 201699. In accordance with 21 U.S.C. § 355(b)(1), the '489 Patent is listed in connection with DIFICID® and NDA No. 201699 in the Orange Book as a patent "with respect to which a claim of patent infringement could reasonably be asserted if a person not licensed by the owner engaged in the manufacture, use, or sale of the drug" DIFICID®.

The '551 Patent

41. The '551 Patent, entitled "18-Membered Macrocycles and Analogs Thereof," was duly and legally issued by the USPTO on November 19, 2013, naming Youe-Kong Shue, Chan-Kou Hwang, Yu-Hung Chiu, Alex Romero, Farah Babakhani, Pamela Sears, and Franklin Okumu as the inventors. A copy of the '551 Patent is attached hereto as Exhibit 2.

42. Plaintiff Merck is the owner, by assignment, of the '551 Patent and has the full right to sue and to recover for infringement thereof. Plaintiff Optimer previously owned the '551 Patent and retains certain interests in the '551 Patent. And Plaintiffs MSD Investment Ireland and MSD International have certain rights in the '551 Patent by license.

43. The '551 Patent is listed in the Orange Book as covering the drug DIFICID®, at the dosage of 200 mg, which is the subject of approved NDA No. 201699. In accordance with 21 U.S.C. § 355(b)(1), the '551 Patent is listed in connection with DIFICID® and NDA No. 201699 in the Orange Book as a patent "with respect to which a claim of patent infringement could reasonably be asserted if a person not licensed by the owner engaged in the manufacture, use, or sale of the drug" DIFICID®.

The '508 Patent

44. The '508 Patent, entitled "Polymorphic Crystalline Forms of Tiacumicin B," was duly and legally issued by the USPTO on May 27, 2008, naming Yu-Hung Chiu, Tessie Mary Che, Alex Romero, Yoshi Ichikawa, and Youe-Kong Shue as the inventors. A copy of the '508 Patent is attached hereto as Exhibit 3.

45. Plaintiff Merck is the owner, by assignment, of the '508 Patent and has the full right to sue and to recover for infringement thereof. Plaintiff Optimer previously owned the '508 Patent and retains certain interests in the '508 Patent. And Plaintiffs MSD Investment Ireland and MSD International have certain rights in the '508 Patent by license.

46. The '508 Patent is listed in the Orange Book as covering the drug DIFICID®, at the dosage of 200 mg, which is the subject of approved NDA No. 201699. In accordance with 21 U.S.C. § 355(b)(1), the '508 Patent is listed in connection with DIFICID® and NDA No. 201699 in the Orange Book as a patent "with respect to which a claim of patent infringement

could reasonably be asserted if a person not licensed by the owner engaged in the manufacture, use, or sale of the drug" DIFICID®.

The '249 Patent

47. The '249 Patent, entitled "Macrolide Polymorphs, Compositions Comprising Such Polymorphs, and Methods of Use and Manufacture Thereof," was duly and legally issued by the USPTO on January 4, 2011, naming Yu-Hung Chiu, Tessie Mary Che, Alex Romero, Yoshi Ichikawa, and Youe-Kong Shue as the inventors. A copy of the '249 Patent is attached hereto as Exhibit 4.

48. Plaintiff Merck is the owner, by assignment, of the '249 Patent and has the full right to sue and to recover for infringement thereof. Plaintiff Optimer previously owned the '249 Patent and retains certain interests in the '249 Patent. And Plaintiffs MSD Investment Ireland and MSD International have certain rights in the '249 Patent by license.

49. The '249 Patent is listed in the Orange Book as covering the drug DIFICID®, at the dosage of 200 mg, which is the subject of approved NDA No. 201699. In accordance with 21 U.S.C. § 355(b)(1), the '249 Patent is listed in connection with DIFICID® and NDA No. 201699 in the Orange Book as a patent "with respect to which a claim of patent infringement could reasonably be asserted if a person not licensed by the owner engaged in the manufacture, use, or sale of the drug" DIFICID®.

The '510 Patent

50. The '510 Patent, entitled "Macrocyclic Polymoprhs, Compositions Comprising Such Polymorphs, and Methods of Use and Manufacture Thereof," was duly and legally issued by the USPTO on October 14, 2014, naming Yu-Hung Chiu, Tessie Mary Che, Alex Romero,

Yoshi Ichikawa, and Youe-Kong Shue as the inventors. A copy of the '510 Patent is attached hereto as Exhibit 5.

51. Plaintiff Merck is the owner, by assignment, of the '510 Patent and has the full right to sue and to recover for infringement thereof. Plaintiff Optimer previously owned the '510 Patent and retains certain interests in the '510 Patent. And Plaintiffs MSD Investment Ireland and MSD International have certain rights in the '510 Patent by license.

52. The '510 Patent is listed in the Orange Book as covering the drug DIFICID®, at the dosage of 200 mg, which is the subject of approved NDA No. 201699. In accordance with 21 U.S.C. § 355(b)(1), the '510 Patent is listed in connection with DIFICID® and NDA No. 201699 in the Orange Book as a patent "with respect to which a claim of patent infringement could reasonably be asserted if a person not licensed by the owner engaged in the manufacture, use, or sale of the drug" DIFICID®.

ACTAVIS'S AMENDED ANDA SUBMISSION

53. By letter dated July 23, 2015 (the "Actavis Notice Letter"), Actavis Labs. FL notified Plaintiffs that it had submitted to the FDA ANDA No. 208443 ("Actavis's ANDA") for Actavis's Fidaxomicin Tablets, a drug product that is a generic copy of DIFICID® (fidaxomicin) Tablets (the "ANDA Product" or "Actavis's ANDA Product").

54. On information and belief, Defendants filed or caused to be filed Actavis's ANDA with the FDA, seeking FDA approval to engage in the commercial manufacture, use, sale, offer for sale, and/or importation of Actavis's ANDA Product prior to the expirations of the '489, the '551, the '508, the '249, and the '510 Patents.

55. In the Actavis Notice Letter, Actavis Labs. FL notified Plaintiffs that, as part of its ANDA No. 208443, Actavis had filed certifications of the type described in 21 U.S.C.

§ 355(j)(2)(A)(vii)(IV) ("Paragraph IV Certification") with respect to the '489, the '551, the '508, the '249, and the '510 Patents. On information and belief, ANDA No. 208443 contains certification(s) pursuant to 21 U.S.C. § 355(j)(2)(A)(vii)(IV) asserting that the '489, the '551, the '508, the '249, and the '510 Patents are invalid, unenforceable and/or will not be infringed by the commercial manufacture, use, sale, offer for sale, or importation of Actavis's ANDA Product.

56. By filing or causing to be filed Actavis's ANDA, Defendants necessarily represented to the FDA that the ANDA Product has the same active ingredient, the same method of administration, the same dosage form, and the same strength as DIFICID® and is bioequivalent to DIFICID®.

57. By letter dated December 6, 2019 (the "Second Actavis Notice Letter"), Actavis Labs. FL notified Plaintiffs that it had submitted to the FDA an amendment to its previously submitted ANDA No. 208443.

58. On information and belief, Defendants filed or caused to be filed Amended ANDA No. 208443 with the FDA, seeking FDA approval to engage in the commercial manufacture, use, sale, offer for sale, and/or importation of Actavis's Fidaxomicin Tablets, a drug product that is a generic copy of DIFICID® (fidaxomicin) Tablets (the "Amended ANDA Product" or "Actavis's Amended ANDA Product") prior to the expirations of the '489, the '551, the '508, the '249, and the '510 Patents.

59. In the Second Actavis Notice Letter, Actavis Labs. FL notified Plaintiffs that, as part of its amendment to ANDA No. 208443, Actavis had filed certifications in accordance with 21 U.S.C. § 355(j)(2)(A)(vii)(IV) ("Paragraph IV Certification") with respect to the '489, the '551, the '508, the '249, and the '510 Patents. On information and belief, Amended ANDA No. 208443 contains certification(s) pursuant to 21 U.S.C. § 355(j)(2)(A)(vii)(IV) asserting that

the '489, the '551, the '508, the '249, and the '510 Patents are invalid, unenforceable and/or will not be infringed by the commercial manufacture, use, sale, offer for sale, or importation of Actavis's Amended ANDA Product.

60. Upon information and belief, Actavis's Paragraph IV Certification in the Amended ANDA is a required "recertification for a previously submitted paragraph IV certification" pursuant to FDA's regulations because Amended ANDA No. 208443 seeks to "add a new indication or other condition of use"; "add a new strength"; "make other than minor changes in product formulation"; and/or seeks to "change the physical form or crystalline structure of the active ingredient." *See* 21 C.F.R. 314.96(d)(1).

61. By filing or causing to be filed the Amended ANDA No. 208443, Defendants necessarily represented to the FDA that the Amended ANDA Product has the same active ingredient, the same method of administration, the same dosage form, and the same strength as DIFICID® and is bioequivalent to DIFICID®. The Second Actavis Notice Letter did not identify any non-infringement basis for claims 1–2, 4–10, and 12–14 of the '489 Patent, claims 1–6 of the '551 Patent, claims 1–2, 8–9, 11–12, and 15–20 of the '508 Patent, claims 1–3, 6, and 10–12 of the '249 Patent, and claims 1–13 of the '510 Patent.

62. On information and belief, if Actavis's Amended ANDA is approved by the FDA, the Defendants will, prior to the expiration of the '489, the '551, the '508, the '249, and the '510 Patents, begin commercially manufacturing, using, selling, offering to sell, and/or importing Actavis's Amended ANDA Product.

63. On information and belief, if Actavis's Amended ANDA is approved by the FDA, the Defendants will, prior to the expiration of the '489, the '551, the '508, the '249, and the '510 Patents, begin marketing Actavis's Amended ANDA Product for the treatment of *Clostridium*

difficile-associated diarrhea in adults 18 years or older, and doctors and patients will use Actavis's Amended ANDA Product for the indications marketed by the Defendants.

64. Defendants had knowledge of the '489, the '551, the '508, the '249, and the '510 Patents at least as of the date when Actavis's original ANDA No. 208443 was submitted to the FDA containing the Paragraph IV Certification with respect to the '489, the '551, the '508, the '249, and the '510 Patents.

65. Defendants' submission to the FDA of Amended ANDA No. 208443, with the Paragraph IV Certification seeking approval to market Actavis's ANDA Product, is an act of infringement by the Defendants of one or more claims of each of the '489, the '551, the '508, the '249, and the '510 Patents under 35 U.S.C. § 271(e)(2). This infringement entitles Plaintiffs to the relief provided by 35 U.S.C. § 271(e)(4), including, *inter alia*, an order of this Court that the effective date of approval for Amended ANDA No. 208443 be a date which is not earlier than the expiration date of the last expiring of the '489, the '551, the '508, the '249, and the '510 Patents, including any extensions of that date.

66. Defendants' anticipated commercial manufacture, use, sale, offer for sale and/or importation of Actavis's Amended ANDA Product will infringe one or more claims of the '489, the '551, the '508, the '249, and the '510 Patents under 35 U.S.C. §§ 271(a), (b), and/or (c).

67. Defendants concede that the submission of the Amended ANDA (and the Amended ANDA Product described therein) infringes one or more claims of each of the '489, the '551, the '508, the '249, and the '510 Patents. The Second Actavis Notice Letter includes a section that purports to provide the factual and legal basis for "Non-Infringement," but Defendants did not identify any non-infringement basis for claims 1–2, 4–10, and 12–14 of the

'489 Patent, claims 1–6 of the '551 Patent, claims 1–2, 8–9, 11–12, and 15–20 of the '508 Patent, claims 1–3, 6, and 10–12 of the '249 Patent, and claims 1–13 of the '510 Patent.

68. This action is being commenced within forty-five days from the date Plaintiffs received the Second Actavis Notice Letter. The Second Actavis Notice Letter was dated December 6, 2019 and was received by Plaintiffs after December 6, 2019.

COUNT I: INFRINGEMENT OF THE '489 PATENT

69. Plaintiffs incorporate by reference each of the preceding paragraphs of this Complaint as if fully set forth herein.

70. The use and/or administration of Actavis's Amended ANDA Product is covered by one or more claims of the '489 Patent.

71. By filing or causing to be filed Amended ANDA No. 208443 under 21 U.S.C. § 355(j) with a Paragraph IV Certification regarding the '489 Patent in order to engage in the commercial manufacture, use, sale, offer for sale, and/or importation of Actavis's Amended ANDA Product before the expiration of the '489 Patent, Defendants committed an act of infringement of one or more claims of the '489 Patent under 35 U.S.C. § 271(e)(2)(A).

72. If Defendants commercially manufacture, use, sell, offer to sell, and/or import the Amended ANDA Product in the United States or import the Amended ANDA Product into the United States, or induce or contribute to any such conduct during the term of the '489 Patent, Defendants would further infringe the '489 Patent under 35 U.S.C. §§ 271(a), (b) and/or (c).

73. The use and/or administration of Actavis's Amended ANDA Product, on information and belief in accordance with and as directed by the proposed labeling for that product, before the expiration of the '489 Patent would infringe one or more claims of the '489 Patent under 35 U.S.C. § 271(a).

74. By seeking approval to distribute the Amended ANDA Product with, on information and belief, its proposed labeling, Defendants intend to cause others, specifically medical professionals, to perform acts that Defendants know will infringe the '489 Patent.

75. Unless enjoined by this Court, Defendants intend to, and will, engage in the infringing commercial manufacture, use, sale, offer for sale, and/or importation of Actavis's Amended ANDA Product immediately and imminently upon approval of Actavis's Amended ANDA.

76. Unless enjoined by this Court, Defendants intend to, and will, actively induce infringement of the '489 Patent when Actavis's Amended ANDA is approved, and intend to, and will do so, immediately and imminently upon approval of Actavis's Amended ANDA.

77. Defendants know that Actavis's Amended ANDA Product and, on information and belief, its proposed labeling are especially made or adapted for use in infringing the '489 Patent, and that Actavis's Amended ANDA Product and, on information and belief, its proposed labeling are not suitable for substantial noninfringing use. Unless enjoined by this Court, Defendants intend to, and will, contribute to the infringement of the '489 Patent immediately and imminently upon approval of Actavis's Amended ANDA.

78. Defendants had knowledge of the '489 Patent at least as of the date Actavis's original ANDA No. 208443 was submitted and are knowingly infringing the '489 Patent.

79. Defendants acted without a reasonable basis for believing that they would not be liable for infringing the '489 Patent, actively inducing infringement of the '489 Patent, and/or contributing to the infringement of the '489 Patent.

80. Unless Defendants are enjoined from infringing the '489 Patent, actively inducing infringement of the '489 Patent, and/or contributing to the infringement of the '489 Patent,

Plaintiffs will suffer irreparable harm for which they have no adequate remedy at law. Pursuant to 35 U.S.C. §§ 271(e)(4)(B) and 283 and Fed. R. Civ. P. 65, a preliminary and permanent injunction should be entered preventing further infringement.

81. Plaintiffs are entitled to the relief provided by 35 U.S.C. § 271(e)(4), including, *inter alia*, an order of this Court that the FDA set the effective date of approval for Amended ANDA No. 208443 to be a date which is not earlier than the expiration date of the '489 Patent, including any extensions of that date.

82. This case is "exceptional," as that term is used in 35 U.S.C. § 285, and Plaintiffs are entitled to an award of their reasonable attorneys' fees and expenses.

COUNT II: INFRINGEMENT OF THE '551 PATENT

83. Plaintiffs incorporate by reference each of the preceding paragraphs of this Complaint as if fully set forth herein.

84. Actavis's Amended ANDA Product is covered by one or more claims of the '551 Patent.

85. By filing or causing to be filed Amended ANDA No. 208443 under 21 U.S.C. § 355(j) with a Paragraph IV Certification regarding the '551 Patent in order to engage in the commercial manufacture, use, sale, offer for sale, and/or importation of Actavis's Amended ANDA Product before the expiration of the '551 Patent, Defendants committed an act of infringement of one or more claims of the '551 Patent under 35 U.S.C. § 271(e)(2)(A).

86. If Defendants commercially manufacture, use, sell, offer to sell, and/or import the Amended ANDA Product in the United States or induce or contribute to any such conduct during the term of the '551 Patent, Defendants would further infringe the '551 Patent under 35 U.S.C. §§ 271(a), (b) and/or (c).

87. The commercial manufacture, use, sale, offer for sale, and/or importation of Actavis's Amended ANDA Product before the expiration of the '551 Patent would infringe one or more claims of the '551 Patent under 35 U.S.C. § 271(a).

88. By seeking approval to distribute the Amended ANDA Product, Defendants intend to cause others, specifically medical professionals and patients, to perform acts that Defendants know will infringe the '551 Patent.

89. Unless enjoined by this Court, Defendants intend to, and will, engage in the infringing commercial manufacture, use, sale, offer for sale, and/or importation of Actavis's Amended ANDA Product immediately and imminently upon approval of Actavis's Amended ANDA.

90. Unless enjoined by this Court, Defendants intend to, and will, actively induce infringement of the '551 Patent when Actavis's Amended ANDA is approved, and intend to, and will do so, immediately and imminently upon approval of Actavis's Amended ANDA.

91. Defendants know that Actavis's Amended ANDA Product is especially made or adapted for use in infringing the '551 Patent, and that Actavis's Amended ANDA Product is not suitable for substantial noninfringing use. Unless enjoined by this Court, Defendants intend to, and will, contribute to the infringement of the '551 Patent immediately and imminently upon approval of Actavis's Amended ANDA.

92. Defendants had knowledge of the '551 Patent at least as of the date Actavis's original ANDA No. 208443 was submitted and are knowingly infringing the '551 Patent.

93. Defendants acted without a reasonable basis for believing that they would not be liable for infringing the '551 Patent, actively inducing infringement of the '551 Patent, and/or contributing to the infringement of the '551 Patent.

94. Unless Defendants are enjoined from infringing the '551 Patent, actively inducing infringement of the '551 Patent, and/or contributing to the infringement of the '551 Patent, Plaintiffs will suffer irreparable harm for which they have no adequate remedy at law. Pursuant to 35 U.S.C. §§ 271(e)(4)(B) and 283 and Fed. R. Civ. P. 65, a preliminary and permanent injunction should be entered preventing further infringement.

95. Plaintiffs are entitled to the relief provided by 35 U.S.C. § 271(e)(4), including, *inter alia*, an order of this Court that the FDA set the effective date of approval for Amended ANDA No. 208443 to be a date which is not earlier than the expiration date of the '551 Patent, including any extensions of that date.

96. This case is "exceptional," as that term is used in 35 U.S.C. § 285, and Plaintiffs are entitled to an award of their reasonable attorneys' fees and expenses.

COUNT III: INFRINGEMENT OF THE '508 PATENT

97. Plaintiffs incorporate by reference each of the preceding paragraphs of this Complaint as if fully set forth herein.

98. Actavis's Amended ANDA Product is covered by one or more claims of the '508 Patent.

99. By filing or causing to be filed Amended ANDA No. 208443 under 21 U.S.C. § 355(j) with a Paragraph IV Certification regarding the '508 Patent in order to engage in the commercial manufacture, use, sale, offer for sale, and/or importation of Actavis's Amended ANDA Product before the expiration of the '508 Patent, Defendants committed an act of infringement of one or more claims of the '508 Patent under 35 U.S.C. § 271(e)(2)(A).

100. If Defendants commercially manufacture, use, sell, offer to sell, and/or import the Amended ANDA Product in the United States or import the Amended ANDA Product into the

United States, or induce or contribute to any such conduct during the term of the '508 Patent, Defendants would further infringe the '508 Patent under 35 U.S.C. §§ 271(a), (b) and/or (c).

101. The commercial manufacture, use, sale, offer for sale, and/or importation of Actavis's Amended ANDA Product before the expiration of the '508 Patent would infringe one or more claims of the '508 Patent under 35 U.S.C. § 271(a).

102. By seeking approval to distribute the Amended ANDA Product, Defendants intend to cause others, specifically medical professionals and patients, to perform acts that Defendants know will infringe the '508 Patent.

103. Unless enjoined by this Court, Defendants intend to, and will, engage in the infringing commercial manufacture, use, sale, offer for sale, and/or importation of Actavis's Amended ANDA Product immediately and imminently upon approval of Actavis's Amended ANDA.

104. Unless enjoined by this Court, Defendants intend to, and will, actively induce infringement of the '508 Patent when Actavis's Amended ANDA is approved, and intend to, and will do so, immediately and imminently upon approval of Actavis's Amended ANDA.

105. Defendants know that Actavis's Amended ANDA Product is especially made or adapted for use in infringing the '508 Patent, and that Actavis's Amended ANDA Product is not suitable for substantial noninfringing use. Unless enjoined by this Court, Defendants intend to, and will, contribute to the infringement of the '508 Patent immediately and imminently upon approval of Actavis's Amended ANDA.

106. Defendants had knowledge of the '508 Patent at least as of the date Actavis's original ANDA No. 208443 was submitted and are knowingly infringing the '508 Patent.

107. Defendants acted without a reasonable basis for believing that they would not be liable for infringing the '508 Patent, actively inducing infringement of the '508 Patent, and/or contributing to the infringement of the '508 Patent.

108. Unless Defendants are enjoined from infringing the '508 Patent, actively inducing infringement of the '508 Patent, and/or contributing to the infringement of the '508 Patent, Plaintiffs will suffer irreparable harm for which they have no adequate remedy at law. Pursuant to 35 U.S.C. §§ 271(e)(4)(B) and 283 and Fed. R. Civ. P. 65, a preliminary and permanent injunction should be entered preventing further infringement.

109. Plaintiffs are entitled to the relief provided by 35 U.S.C. § 271(e)(4), including, *inter alia*, an order of this Court that the FDA set the effective date of approval for Amended ANDA No. 208443 to be a date which is not earlier than the expiration date of the '508 Patent, including any extensions of that date.

110. This case is "exceptional," as that term is used in 35 U.S.C. § 285, and Plaintiffs are entitled to an award of their reasonable attorneys' fees and expenses.

COUNT IV: INFRINGEMENT OF THE '249 PATENT

111. Plaintiffs incorporate by reference each of the preceding paragraphs of this Complaint as if fully set forth herein.

112. Actavis's Amended ANDA Product is covered by one or more claims of the '249 Patent.

113. By filing or causing to be filed Amended ANDA No. 208443 under 21 U.S.C. § 355(j) with a Paragraph IV Certification regarding the '249 Patent in order to engage in the commercial manufacture, use, sale, offer for sale, and/or importation of Actavis's Amended

ANDA Product before the expiration of the '249 Patent, Defendants committed an act of infringement of one or more claims of the '249 Patent under 35 U.S.C. § 271(e)(2)(A).

114. If Defendants commercially manufacture, use, sell, offer to sell, and/or import the Amended ANDA Product in the United States or import the Amended ANDA Product into the United States, or induce or contribute to any such conduct during the term of the '249 Patent, Defendants would further infringe the '249 Patent under 35 U.S.C. §§ 271(a), (b) and/or (c).

115. The commercial manufacture, use, sale, offer for sale, and/or importation of Actavis's Amended ANDA Product before the expiration of the '249 Patent would infringe one or more claims of the '249 Patent under 35 U.S.C. § 271(a).

116. By seeking approval to distribute the Amended ANDA Product, Defendants intend to cause others, specifically medical professionals and patients, to perform acts that Defendants know will infringe the '249 Patent.

117. Unless enjoined by this Court, Defendants intend to, and will, engage in the infringing commercial manufacture, use, sale, offer for sale, and/or importation of Actavis's Amended ANDA Product immediately and imminently upon approval of Actavis's Amended ANDA.

118. Unless enjoined by this Court, Defendants intend to, and will, actively induce infringement of the '249 Patent when Actavis's Amended ANDA is approved, and intend to, and will do so, immediately and imminently upon approval of Actavis's Amended ANDA.

119. Defendants know that Actavis's Amended ANDA Product is especially made or adapted for use in infringing the '249 Patent, and that Actavis's Amended ANDA Product is not suitable for substantial noninfringing use. Unless enjoined by this Court, Defendants intend to,

and will, contribute to the infringement of the '249 Patent immediately and imminently upon approval of Actavis's Amended ANDA.

120. Defendants had knowledge of the '249 Patent at least as of the date Actavis's original ANDA No. 208443 was submitted and are knowingly infringing the '249 Patent.

121. Defendants acted without a reasonable basis for believing that they would not be liable for infringing the '249 Patent, actively inducing infringement of the '249 Patent, and/or contributing to the infringement of the '249 Patent.

122. Unless Defendants are enjoined from infringing the '249 Patent, actively inducing infringement of the '249 Patent, and/or contributing to the infringement of the '249 Patent, Plaintiffs will suffer irreparable harm for which they have no adequate remedy at law. Pursuant to 35 U.S.C. §§ 271(e)(4)(B) and 283 and Fed. R. Civ. P. 65, a preliminary and permanent injunction should be entered preventing further infringement.

123. Plaintiffs are entitled to the relief provided by 35 U.S.C. § 271(e)(4), including, *inter alia*, an order of this Court that the FDA set the effective date of approval for Amended ANDA No. 208443 to be a date which is not earlier than the expiration date of the '249 Patent, including any extensions of that date.

124. This case is "exceptional," as that term is used in 35 U.S.C. § 285, and Plaintiffs are entitled to an award of their reasonable attorneys' fees and expenses.

COUNT V: INFRINGEMENT OF THE '510 PATENT

125. Plaintiffs incorporate by reference each of the preceding paragraphs of this Complaint as if fully set forth herein.

126. The use and/or administration of Actavis's Amended ANDA Product is covered by one or more claims of the '510 Patent.

127. By filing or causing to be filed Amended ANDA No. 208443 under 21 U.S.C. § 355(j) with a Paragraph IV Certification regarding the '510 Patent in order to engage in the commercial manufacture, use, sale, offer for sale, and/or importation of Actavis's Amended ANDA Product before the expiration of the '510 Patent, Defendants committed an act of infringement of one or more claims of the '510 Patent under 35 U.S.C. § 271(e)(2)(A).

128. If Defendants commercially manufacture, use, sell, offer to sell, and/or import the Amended ANDA Product in the United States or import the Amended ANDA Product into the United States, or induce or contribute to any such conduct during the term of the '510 Patent, Defendants would further infringe the '510 Patent under 35 U.S.C. §§ 271(a), (b) and/or (c).

129. The use and/or administration of Actavis's Amended ANDA Product, on information and belief in accordance with and as directed by the proposed labeling for that product, before the expiration of the '510 Patent would infringe one or more claims of the '510 Patent under 35 U.S.C. § 271(a).

130. By seeking approval to distribute the Amended ANDA Product with, on information and belief, its proposed labeling, Defendants intend to cause others, specifically medical professionals, to perform acts that Defendants know will infringe the '510 Patent.

131. Unless enjoined by this Court, Defendants intend to, and will, engage in the infringing commercial manufacture, use, sale, offer for sale, and/or importation of Actavis's Amended ANDA Product immediately and imminently upon approval of Actavis's Amended ANDA.

132. Unless enjoined by this Court, Defendants intend to, and will, actively induce infringement of the '510 Patent when Actavis's Amended ANDA is approved, and intend to, and will do so, immediately and imminently upon approval of Actavis's Amended ANDA.

133. Defendants know that Actavis's Amended ANDA Product and, on information and belief, its proposed labeling are especially made or adapted for use in infringing the '510 Patent, and that Actavis's Amended ANDA Product and, on information and belief, its proposed labeling are not suitable for substantial noninfringing use. Unless enjoined by this Court, Defendants intend to, and will, contribute to the infringement of the '510 Patent immediately and imminently upon approval of Actavis's Amended ANDA.

134. Defendants had knowledge of the '510 Patent at least as of the date Actavis's original ANDA No. 208443 was submitted and are knowingly infringing the '510 Patent.

135. Defendants acted without a reasonable basis for believing that they would not be liable for infringing the '510 Patent, actively inducing infringement of the '510 Patent, and/or contributing to the infringement of the '510 Patent.

136. Unless Defendants are enjoined from infringing the '510 Patent, actively inducing infringement of the '510 Patent, and/or contributing to the infringement of the '510 Patent, Plaintiffs will suffer irreparable harm for which they have no adequate remedy at law. Pursuant to 35 U.S.C. §§ 271(e)(4)(B) and 283 and Fed. R. Civ. P. 65, a preliminary and permanent injunction should be entered preventing further infringement.

137. Plaintiffs are entitled to the relief provided by 35 U.S.C. § 271(e)(4), including, *inter alia*, an order of this Court that the FDA set the effective date of approval for Amended ANDA No. 208443 to be a date which is not earlier than the expiration date of the '510 Patent, including any extensions of that date.

138. This case is "exceptional," as that term is used in 35 U.S.C. § 285, and Plaintiffs are entitled to an award of their reasonable attorneys' fees and expenses.

PRAYER FOR RELIEF

WHEREFORE, Plaintiffs respectfully request the following relief:

A. Judgment in favor of Plaintiffs and against Defendants;

B. Judgment that the '489, the '551, the '508, the '249, and the '510 Patents have not been proven invalid or unenforceable.

C. Judgment that the Defendants have infringed, literally or by the doctrine of equivalents, the '489, the '551, the '508, the '249, and the '510 Patents under 35 U.S.C. § 271(e)(2) by the submission of Amended ANDA No. 208443;

D. Judgment declaring that commercial manufacturing, using, selling, offering to sell, and/or importing Actavis's Amended ANDA Product, or inducing or contributing to such conduct, will constitute infringement, active inducement of infringement and/or contributory infringement of the '489, the '551, the '508, the '249, and the '510 Patents by Defendants under 35 U.S.C. §§ 271(a), (b) and/or (c);

E. Judgment, pursuant to 35 U.S.C. § 271(e)(4)(A), that the effective date of any FDA approval of Amended ANDA No. 208443 under § 505(j) of the Federal Food, Drug and Cosmetic Act (21 U.S.C. § 355(j)) shall be a date no earlier than the date of expiration of the last expiring of the '489, the '551, the '508, the '249, and the '510 Patents plus any additional periods of exclusivity to which the Patents are or become entitled;

F. A preliminary and permanent injunction, pursuant to 35 U.S.C. §§ 271(e)(4)(B) and 283 and Fed. R. Civ. P. 65 enjoining Defendants, and their officers, partners, agents, servants, employees, parents, subsidiaries, divisions, affiliate corporations, other related business entities and all other persons acting in concert, participation, or in privity with them, and their successors and assigns, from any commercial manufacture, use, sale, offer to sell, and/or importation within the United States of any drug product described in Amended ANDA No. 208443, and any product that is similar to or only colorably different from those products,

before the date of expiration of the last expiring of the '489, the '551, the '508, the '249, and the '510 Patents plus any additional periods of exclusivity to which the Patents are or become entitled;

G. Damages or other monetary relief, including prejudgment and postjudgment interest, if Defendants engage in the commercial manufacture, use, sale, offer to sell, or importation of Actavis's Amended ANDA Product, or any other products that infringe the '489, the '551, the '508, the '249, and the '510 Patents, or that induce or contribute to the infringement of the '489, the '551, the '508, the '249, and the '510 Patents, prior to the expiration of the last expiring of the '489, the '551, the '508, the '249, and the '510 Patents plus any additional periods of exclusivity to which the Patents are or become entitled;

H. A declaration that this an exceptional case and an award to Plaintiffs of their reasonable attorneys' fees and expenses, as provided by 35 U.S.C. §§ 271(e)(4) and 285; and

I. Such other and further relief as this Court may deem just and proper.

Dated: January 16, 2020

Respectfully submitted,

By: s/ Charles M. Lizza

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CERTIFICATION PURSUANT TO LOCAL CIVIL RULES 11.2 & 40.1

I hereby certify that, to the best of my knowledge, the matter captioned *Merck Sharp & Dohme Corp., et al. v. Actavis Labs. FL, Inc.*, Civil Action No. 15-6541 (CCC)(CLW) is related to the matter in controversy because the matter in controversy involves an amendment to the original ANDA that is the subject of Civil Action No. 15-6541, the same Plaintiffs, some of the same Defendants, and the same patents.

I further certify that, to the best of my knowledge, the matter in controversy is not the subject of any other action pending in any court, or of any pending arbitration or administrative proceeding.

Dated: January 16, 2020

Respectfully submitted,

By: s/ Charles M. Lizza

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EXHIBIT 1

US007906489B2

(12) **United States Patent**
Shue et al.

(10) **Patent No.: US 7,906,489 B2**
(45) **Date of Patent: Mar. 15, 2011**

(54) **18-MEMBERED MACROCYCLES AND ANALOGS THEREOF**

(75) Inventors: **Youe-Kong Shue**, Carlsbad, CA (US); **Chan-Kou Hwang**, San Diego, CA (US); **Yu-Hung Chiu**, San Diego, CA (US); **Alex Romero**, San Diego, CA (US); **Farah Babakhani**, San Diego, CA (US); **Pamela Sears**, San Diego, CA (US); **Franklin Okumu**, Oakland, CA (US)

(73) Assignee: **Optimer Pharmaceuticals, Inc.**, San Diego, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 762 days.

(21) Appl. No.: **11/882,219**

(22) Filed: **Jul. 31, 2007**

(65) **Prior Publication Data**

US 2008/0269145 A1 Oct. 30, 2008

Related U.S. Application Data

(63) Continuation-in-part of application No. PCT/US2005/002887, filed on Jan. 31, 2005.

(60) Provisional application No. 60/570,697, filed on May 14, 2004.

(51) **Int. Cl.**
A01N 43/04 (2006.01)
A61K 31/70 (2006.01)

(52) **U.S. Cl.** **514/28**; 514/867

(58) **Field of Classification Search** 514/28, 514/867

See application file for complete search history.

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(Continued)

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Assistant Examiner — Scarlett Goon
(74) *Attorney, Agent, or Firm* — Morgan Lewis & Bockius LLP

(57) **ABSTRACT**

The present invention relates generally to the 18-membered macrocyclic antimicrobial agents called Tiacumicins, specifically, OPT-80 (which is composed almost entirely of the R-Tiacumicin B), pharmaceutical compositions comprising OPT-80, and methods using OPT-80. In particular, this compound is a potent drug for the treatment of bacterial infections, specifically *C. difficile* infections.

14 Claims, 1 Drawing Sheet

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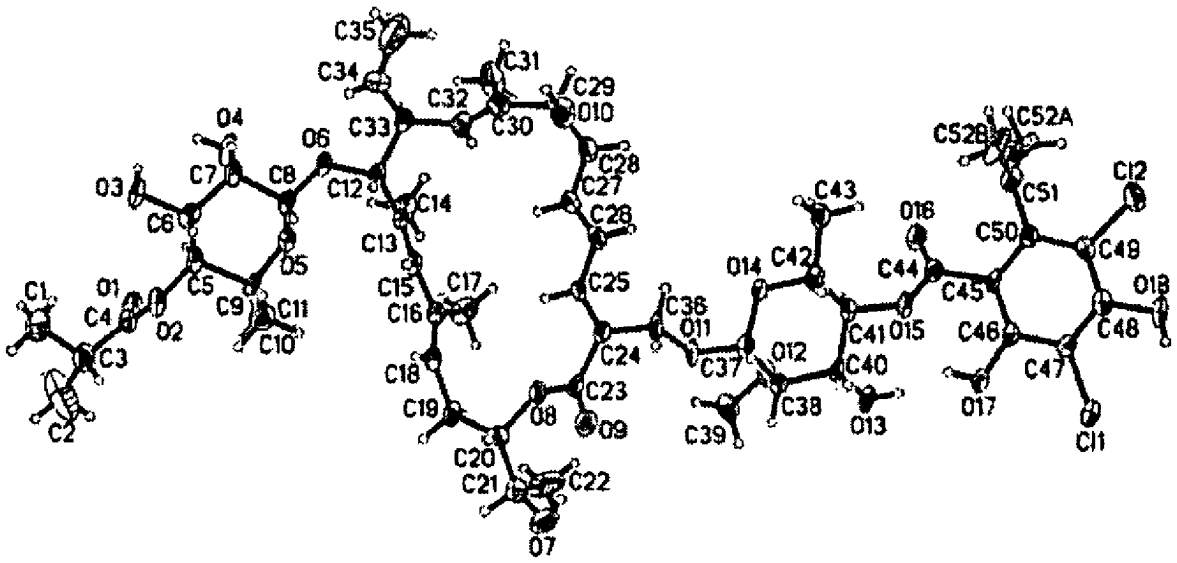
Figure 1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C40
C46
C48
O18
C47
O17
O13
C51
C52B
C52A
Cl2
Cl1

18-MEMBERED MACROCYCLES AND ANALOGS THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application is a continuation-in-part application of International Application PCT/US2005/002887 filed Jan. 31, 2005, and claims priority to U.S. Provisional Application No. 60/570,697 filed May 14, 2004, each application of which is incorporated by reference in its entirety.

FIELD OF INVENTION

The present invention relates generally to the 18-membered macrocyclic antimicrobial agents called Tiacumicins, specifically, the R-Tiacumicin B or Tiacumicin B and its related compounds. In particular, substantially pure R-Tiacumicin B, as a potent antibiotic agent for the treatment of bacterial infections, specifically GI infections caused by toxin producing strains of *Clostridium difficile* (*C. difficile*), *Staphylococcus aureus* (*S. aureus*) including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Clostridium perfringens* (*C. perfringens*).

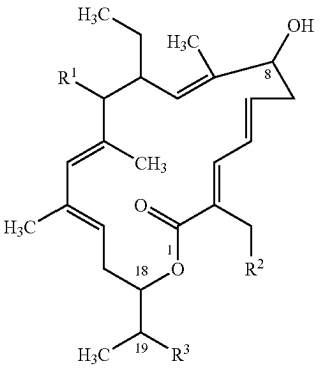
BACKGROUND OF THE INVENTION

Macrocycles are an important therapeutic class of antibiotics. These compounds are frequently produced as a family of closely related biogenetic congeners. The Tiacumicins are a series of 18-membered macrocyclic antibiotics in which the

macrocyclic ring is glycosidically attached to one or two sugars. A seven-carbon sugar is esterfied at various positions with small fatty acids. The other sugar, when present, is esterified with an isomer of the fully substituted benzoic acid, everninic acid. (Journal of Liquid Chromatography, 1988, 11: 191-201).

Tiacumicins are a family of related compounds that contain the 18-membered ring shown in Formula I below.

Formula I



At present, several distinct Tiacumicins have been identified and six of these (Tiacumicin A-F) are defined by their particular pattern of substituents R^1, R^2, and R^3 (U.S. Pat. No. 4,918,174; J. Antibiotics, 1987, 40: 575-588), as shown in Table 1.

TABLE 1

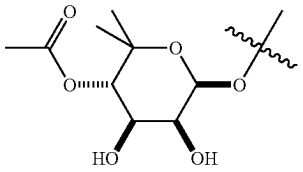
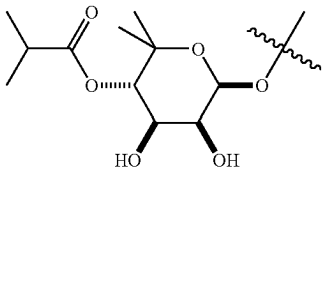
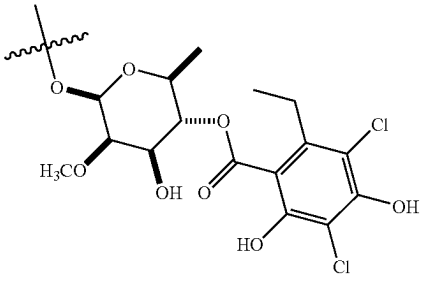
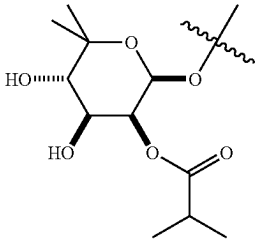
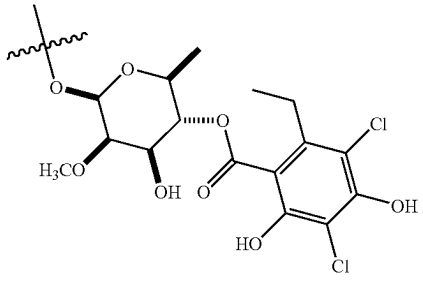
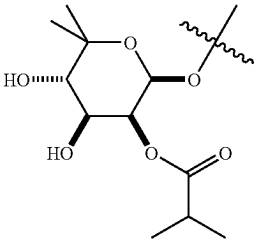
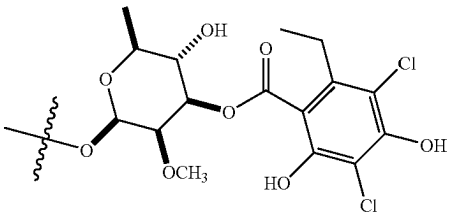
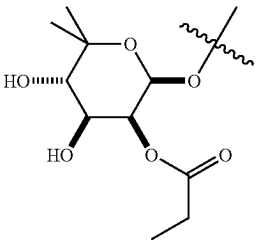
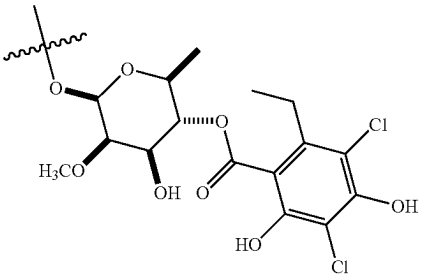
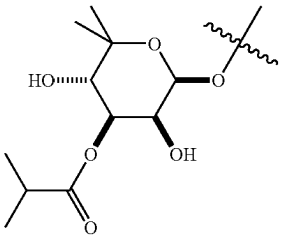
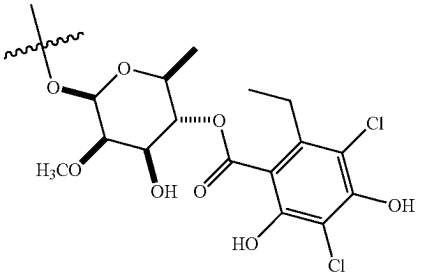
Substituents Present In Tiacumcins A-F			
	R^1	R^2	R^3
A		H	H
B			OH
C			OH

TABLE 1-continued

Substituents Present In Tiacumcins A-F

	R^1	R^2	R^3
D			OH
E			OH
F			OH

Tiacumicins A-F have been characterized spectroscopically and by other physical methods. The chemical structures of Tiacumicins are based on spectroscopy: UV-vis, IR and ^1H and ^{13}C NMR, see for example J. Antibiotics, 1987, 40: 575-588. Inspection of Table 1 reveals that certain members of the family are structurally related isomers and/or differ by the presence or absence of certain moieties. Others differ in the nature of their ester groups.

Tiacumicins are produced by bacteria, including *Dactylosporangium aurantiacum* subspecies *hamdenensis*, which may be obtained from the ARS Patent Collection of the Northern Regional Research Center, United States Department of Agriculture, 1815 North University Street, Peoria, Ill. 61604, accession number NRRL 18085. The characteristics of strain AB 718C-41 are given in J. Antibiotics, 1987, 40: 567-574 and U.S. Pat. No. 4,918,174.

C. difficile-associated diarrhea (CDAD) is a disease characterized by severe and painful diarrhea. *C. difficile* is responsible for approximately 20% of the cases of antibiotic-associated diarrhea (AAD) and the majority of the cases of antibiotic-associated colitis (AAC). These diseases are typically caused by toxin producing strains of *C. difficile, S. aureus* including methicillin-resistant *S. aureus* (MRSA) and *Clostridium perfringens* (*C. perfringens*). AAD represents a major economic burden to the healthcare system that is conservatively estimated at $3-6 billion per year in excess hospital costs in the U.S. alone.

Vancomycin-resistant enterococci, for which intestinal colonization provides a constant reservoir for infection, has also emerged as a major nosocomial pathogen associated with increased health care cost and mortality. VRE can appear as coinfection in patients infected with *C. difficile*, or more commonly cause infection in certain high risk patients such as haematology and oncology patients, patients in intensive care units and patients receiving solid organ transplants.

Methicillin-resistant Staphylococci, such as MRSA, are increasing in prevalence in both the hospital and community settings. Staphylococci are found on the skin and within the digestive and respiratory tracts but can infect open wounds and burns and can progress to serious systemic infection. The emergence of multi-drug resistant Staphylococci, especially, in the hospital where antibiotic use is frequent and selective pressure for drug-resistant organisms is high, has proven a challenge for treating these patients. The presence of MRSA on the skin of patients and health care workers promotes transmission of the multi-drug resistant organisms.

Similar diseases, including but not limited to clostridial enterocolitis, neonatal diarrhea, antibiotic-associated enterocolitis, sporadic enterocolitis, and nosocomial enterocolitis are also significant problems in some animal species.

AAD is a significant problem in hospitals and long-term care facilities and in the community. *C. difficile* is the leading cause of AAD in the hospital setting, accounting for approximately 20% of cases of AAD and the majority of cases of

antibiotic-associated colitis (AAC). The rising incidence of *Clostridium difficile*-associated diarrhea (CDAD) has been attributed to the frequent prescription of broad-spectrum antibiotics to hospitalized patients.

The most serious form of the disease is pseudomembranous colitis (PMC), which is manifested histologically by colitis with mucosal plaques, and clinically by severe diarrhea, abdominal cramps, and systemic toxicity. The overall mortality rate from CDAD is low, but is much greater in patients who develop severe colitis or systemic toxicity. A recent study has shown that even when death is not directly attributable to *C. difficile*, the rate of mortality in CDAD patients as compared to case-matched controls is much greater.

Diarrhea and colitis are caused by the elaboration of one or more *C. difficile* toxins. The organism proliferates in the colon in patients who have been given broad-spectrum antibiotics or, less commonly, cancer chemotherapy. CDAD is diagnosed in approximately 20% of hospitalized patients who develop diarrhea after treatment with such agents.

There are currently two dominant therapies for CDAD: vancomycin and metronidazole. Vancomycin is not recommended for first-line treatment of CDAD mainly because it is the only antibiotic active against some serious life-threatening multi-drug resistant bacteria. Therefore, in an effort to minimize the emergence of vancomycin-resistant *Enterococcus* (VRE) or vancomycin-resistant *S. aureus* (VRSA), the medical community discourages the use of this drug except when absolutely necessary.

Metronidazole is recommended as initial therapy out of concern for the promotion and selection of vancomycin resistant gut flora, especially enterococci. Despite reports that the frequency of *C. difficile* resistance may be >6% in some countries, metronidazole remains nearly as effective as vancomycin, is considerably less expensive, and can be used either orally or intravenously. Metronidazole is associated with significant adverse effects including nausea, neuropathy, leukopenia, seizures, and a toxic reaction to alcohol. Furthermore, it is not safe for use in children or pregnant women. Clinical recurrence occurs in up to 20% of cases after treatment with either vancomycin or metronidazole. Therapy with metronidazole has been reported to be an important risk factor for VRE colonization and infection. The current treatment regime against Gastrointestinal infections, e.g., *Clostridium difficile*-associated diarrhea (CDAD) is rather cumbersome, requiring up to 500 mg four-times daily for 10 to 14 days. Thus, there is a need for better treatment for cases of CDAD as well as for cases of other Antibiotic-associated diarrhea (AAD) and Antibiotic-associated colitis (AAC).

Tiacumicins, specifically Tiacumicin B, show activity against a variety of bacterial pathogens and in particular against *C. difficile*, a Gram-positive bacterium (Antimicrob. Agents Chemother. 1991, 1108-1111). *C. difficile* is an anaerobic spore-forming bacterium that causes an infection of the bowel. Diarrhea is the most common symptom but abdominal pain and fever may also occur. *C. difficile* is a major causative agent of colitis (inflammation of the colon) and diarrhea that may occur following antibiotic intake. This bacterium is primarily acquired in hospitals and chronic care facilities. Because Tiacumicin B shows promising activity against *C. difficile*, it is expected to be useful in the treatment of bacterial infections, especially those of the gastrointestinal tract, in mammals. Examples of such treatments include but are not limited to treatment of colitis and treatment of irritable bowel syndrome. Tiacumicins may also find use for the treatment of gastrointestinal cancers.

Tiacumicin antibiotics are described in U.S. Pat. No. 4,918,174 (issued Apr. 17, 1990), J. Antibiotics 1987, 40: 575-588, J. Antibiotics 1987, 40: 567-574, J. Liquid Chromatography 1988, 11: 191-201, Antimicrobial Agents and Chemotherapy 1991, 35: 1108-1111, U.S. Pat. No. 5,583,115 (issued Dec. 10, 1996), and U.S. Pat. No. 5,767,096 (issued Jun. 16, 1998), which are all incorporated herein by reference. Related compounds are the Lipiarmycin antibiotics (c.f., J. Chem. Soc. Perkin Trans. I, 1987, 1353-1359 and J. Antibiotics 1988, 41: 308-315) and the Clostomicin antibiotics (J. Antibiotics 1986, 39: 1407-1412), which are all incorporated herein by reference.

SUMMARY OF THE INVENTION

The present invention relates to new pharmaceutical compositions containing R-Tiacumicins, specifically the optically pure R-Tiacumicin B, and to the use of these new compositions in combination with existing drugs to treat infections caused by gram-positive anerobes.

One embodiment of the present invention is directed towards the discovery that the chiral center at C-19 of Tiacumicin B has great effect on biological activity. It has now been discovered that a substantially pure preparation of higher activity R-Tiacumicin B, which has an R-hydroxy group at C-19 has surprisingly lower MIC values than the optically pure S-isomer of Tiacumicin B and other Tiacumicin B related compounds.

In another embodiment of the present invention the substantially pure R-Tiacumicin B has an unusually long post-antibiotic activity (PAE).

This invention encompasses the composition of novel antibiotic agents, containing substantially pure R-Tiacumicins, by submerged aerobic fermentation of the microorganism *Dactylosporangium aurantiacum* subspecies *hamdenensis*. The production method is covered by WO 2004/014295 A2, which is hereby incorporated by reference.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** shows the Oak Ridge Thermal Ellipsoid Plot Program (ORTEP) chemical structure of R-Tiacumicin B.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

The term "antibiotic-associated condition" refers to a condition resulting when antibiotic therapy disturbs the balance of the microbial flora of the gut, allowing pathogenic organisms such as enterotoxin producing strains of *C. difficile, S. aureus* and *C. perfringens* to flourish. These organisms can cause diarrhea, pseudomembranous colitis, and colitis and are manifested by diarrhea, urgency, abdominal cramps, tenesmus, and fever among other symptoms. Diarrhea, when severe, causes dehydration and the medical complications associated with dehydration.

The term "asymmetrically substituted" refers to a molecular structure in which an atom having four tetrahedral valences is attached to four different atoms or groups. The commonest cases involve the carbon atom. In such cases, two optical isomers (D- and L-enantiomers or R- and S-enantiomers) per carbon atom result which are nonsuperposable

mirror images of each other. Many compounds have more than one asymmetric carbon. This results in the possibility of many optical isomers, the number being determined by the formula 2^n, where n is the number of asymmetric carbons.

As used herein, and unless otherwise indicated, the terms "biohydrolyzable carbamate," "biohydrolyzable carbonate," "biohydrolyzable ureide" and "biohydrolyzable phosphate" mean a carbamate, carbonate, ureide and phosphate, respectively, of a compound that either: 1) does not interfere with the biological activity of the compound but can confer upon that compound advantageous properties in vivo, such as uptake, duration of action, or onset of action; or 2) is biologically inactive but is converted in vivo to the biologically active compound. Examples of biohydrolyzable carbamates include, but are not limited to, lower alkylamines, substituted ethylenediamines, aminoacids, hydroxyalkylamines, heterocyclic and heteroaromatic amines, and polyether amines.

As used herein, and unless otherwise indicated, the term "biohydrolyzable ester" means an ester of a compound that either: 1) does not interfere with the biological activity of the compound but can confer upon that compound advantageous properties in vivo, such as uptake, duration of action, or onset of action; or 2) is biologically inactive but is converted in vivo to the biologically active compound. Examples of biohydrolyzable esters include, but are not limited to, lower alkyl esters, alkoxyacyloxy esters, alkyl acylamino alkyl esters, and choline esters.

As used herein, and unless otherwise indicated, the term "biohydrolyzable amide" means an amide of a compound that either: 1) does not interfere with the biological activity of the compound but can confer upon that compound advantageous properties in vivo, such as uptake, duration of action, or onset of action; or 2) is biologically inactive but is converted in vivo to the biologically active compound. Examples of biohydrolyzable amides include, but are not limited to, lower alkyl amides, .alpha.-amino acid amides, alkoxyacyl amides, and alkylaminoalkylcarbonyl amides.

The term "broth" as used herein refers to the fluid culture medium as obtained during or after fermentation. Broth comprises a mixture of water, the desired antibiotic(s), unused nutrients, living or dead organisms, metabolic products, and the adsorbent with or without adsorbed product.

The term "C-19 Ketone" refers to a Tiacumicin B related compound shown below in Formula II:

The term "diastereomers" refers to stereoisomers that are not mirror images of each other.

The term "enantiomer" refers to a non-superimposable mirror image of itself. An enantiomer of an optically active isomer rotates plane polarized light in an equal but opposite direction of the original isomer. A solution of equal parts of an optically active isomer and its enantiomer is known as a racemic solution and has a net rotation of plane polarized light of zero. Enantiomers will have the opposite prefixes of each other: D- becomes L- or R- becomes S-. Often only one enantiomer is active in a biological system, because most biological reactions are enzymatic and the enzymes can only attach to one of the enantiomers.

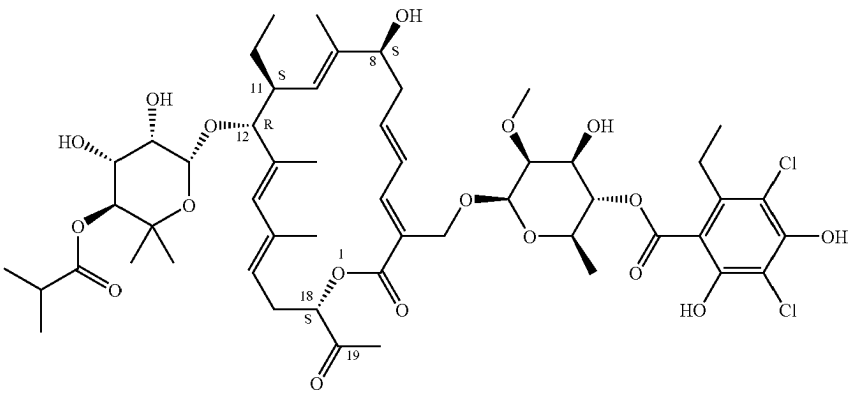
The term "excipient" refers to an inert substance added to a pharmacological composition to further facilitate administration of a compound. Examples of excipients include but are not limited to, calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

The term "halogen" includes F, Cl, Br and I.

As used herein, the term "hydrate" means a compound of the present invention or a salt thereof that further includes a stoichiometric or non-stoichiometric amount of water bound by non-covalent intermolecular forces.

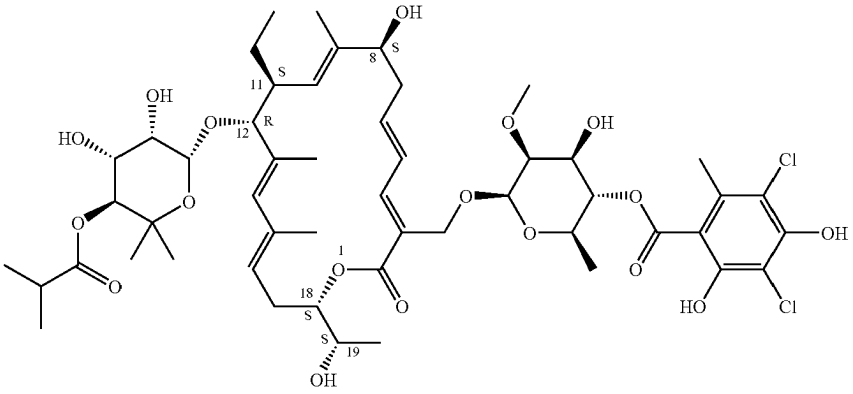
The term "isomeric mixture" means a mixture of two or more configurationally distinct chemical species having the same chemical formula. An isomeric mixture is a genus comprising individual isomeric species. Examples of isomeric mixtures include stereoisomers (enantiomers and diastereomers), regioisomers, as might result for example from a pericyclic reaction. The compounds of the present invention comprise asymmetrically substituted carbon atoms. Such asymmetrically substituted carbon atoms can result in mixtures of stereoisomers at a particular asymmetrically substituted carbon atom or a single stereoisomer. As a result, racemic mixtures, mixtures of diastereomers, as well as single diastereomers of the compounds of the invention are included in the present invention.

Formula II



The term "Lipiarmycin A4" refers to a Tiacumicin B related compound shown below in Formula III:

Formula III



The term "lower alkyl," alone or in combination, refers to an optionally substituted straight-chain or optionally substituted branched-chain having from 1 to about 8 carbons (e.g., C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8), more preferably 1 to 4 carbons (e.g., C_1, C_2, C_3, C_4). Examples of alkyl radicals include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl. A "lower alkyl" is generally a shorter alkyl, e.g., one containing from 1 to about 4 carbon atoms (e.g., C_1, C_2, C_3, C_4).

The term "macrocycles" refers to organic molecules with large ring structures usually containing over 10 atoms.

The term "18-membered macrocycles" refers to organic molecules with ring structures containing 18 atoms.

The term "membered ring" can embrace any cyclic structure, including carbocycles and heterocycles as described above. The term "membered" is meant to denote the number of skeletal atoms that constitute the ring. Thus, for example, pyridine, pyran and thiopyran are 6 membered rings and pyrrole, furan, and thiophene are 5 membered rings.

The term "MIC" or "minimum inhibitory concentration" refers to the lowest concentration of an antibiotic that is needed to inhibit growth of a bacterial isolate in vitro. A common method for determining the MIC of an antibiotic is to prepare several tubes containing serial dilutions of the antibiotic, that are then inoculated with the bacterial isolate of interest. The MIC of an antibiotic can be determined from the tube with the lowest concentration that shows no turbidity (no growth).

The term "MIC_{50}" refers to the lowest concentration of antibiotic required to inhibit the growth of 50% of the bacterial strains tested within a given bacterial species.

The term "MIC_{90}" refers to the lowest concentration of antibiotic required to inhibit the growth of 90% of the bacterial strains tested within a given bacterial species.

The term "OPT-80" refers to a preparation containing R-Tiacumicin B and Tiacumicin B related compounds (including, but not limited to, Tiacumicins, Lipiarmycin A4 and C-19 Ketone). Preparations of this type are described in detail in PCT application PCT/US03/21977, having an international publication number of WO 2004/014295 A2 and which preparations and are incorporated here by reference.

The term "ORTEP" refers to the Oak Ridge Thermal Ellipsoid Plot computer program, written in Fortran, for drawing crystal structure illustrations. Ball-and-stick type illustrations of a quality suitable for publication are produced with either spheres or thermal-motion probability ellipsoids, derived from anisotropic temperature factor parameters, on the atomic sites. The program also produces stereoscopic pairs of illustrations which aid in the visualization of complex arrangements of atoms and their correlated thermal motion patterns.

The term "PAE" or "post-antibiotic effect" refers to a well-established pharmacodynamic parameter that reflects the persistent suppression of bacterial growth following antibiotic exposure.

The term "patient" refers to a human or animal in need of medical treatment. For the purposes of this invention, human patients are typically institutionalized in a primary medical care facility such as a hospital or nursing home. However, treatment of a disease associated with the use of antibiotics or cancer chemotherapies or antiviral therapies can occur on an outpatient basis, upon discharge from a primary care facility, or can be prescribed by a physician for home-care, not in association with a primary medical care facility. Animals in need of medical treatment are typically in the care of a veterinarian.

The term "pharmaceutically acceptable carrier" refers to a carrier or diluent that is pharmaceutically acceptable.

The term "pharmaceutically acceptable salts" refers to those derived from pharmaceutically acceptable inorganic and organic bases. Salts derived from appropriate bases include alkali metal (e.g., sodium or potassium), alkaline earth metal (e.g., magnesium), ammonium and $N(C_1\text{-}C_4\ alkyl)_4^+$ salts, and the like. Illustrative examples of some of these include sodium hydroxide, potassium hydroxide, choline hydroxide, sodium carbonate, and the like. The term "pharmaceutically acceptable salt" also refers to salts prepared from pharmaceutically acceptable non-toxic acids, including inorganic acids and organic acids. Suitable non-toxic acids include inorganic and organic acids such as, but not limited to, acetic, alginic, anthranilic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethenesulfonic, formic, fumaric, furoic, gluconic, glutamic, glucorenic, galacturonic, glycidic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phenylacetic, propionic, phosphoric, salicylic, stearic, succinic, sulfanilic, sulfuric, tartaric acid, p-toluenesulfonic and the like. Particularly preferred are hydrochloric, hydrobromic, phosphoric, and sulfuric acids, and most particularly preferred is the hydrochloride salt.

The term "pharmaceutical composition" refers to a composition of the R-Tiacumicin described herein, or physiologi-

cally acceptable salts thereof, with other chemical components, such as physiologically acceptable carriers and/or excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to a mammal, including humans.

The term "physiologically acceptable carrier" refers to a carrier or diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound.

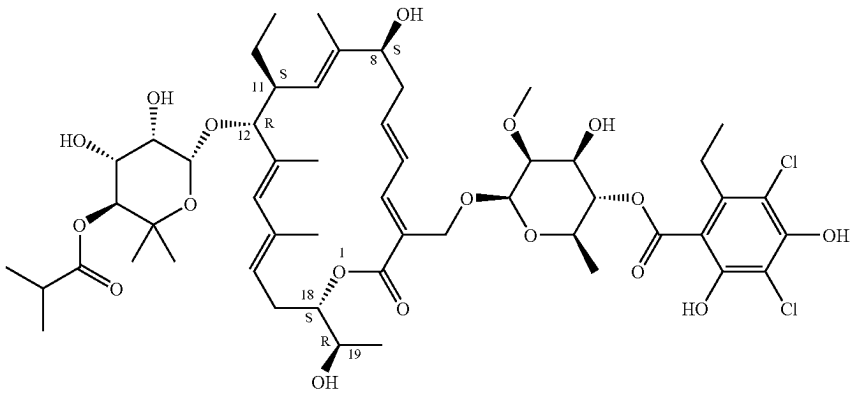
As used herein, and unless otherwise indicated, the term "prodrug" means a derivative of a compound that can hydrolyze, oxidize, or otherwise react under biological conditions (in vitro or in vivo) to provide the compound. Examples of prodrugs include, but are not limited to, compounds that comprise biohydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. Other examples of prodrugs include compounds that comprise —NO, —NO_2, —ONO, or —ONO_2 moieties. When used to describe a compound of the invention, the term "prodrug" may also to be interpreted to exclude other compounds of the invention for example racemates.

The term "pseudomembranous colitis" or "enteritis" refers to the formation of pseudomembranous material (i.e., material composed of fibrin, mucous, necrotic epithelial cells and leukocytes) due to inflammation of the mucous membrane of both the small and large intestine.

The terms "R" and "S" configuration, as used herein, are as defined by the IUPAC 1974 Recommendations for Section E, Fundamental Stereochemistry, *Pure Appl. Chem.* (1976) 45, 13-30. Chiral molecules can be named based on the atomic numbers of the atoms or groups of atoms, the ligands that are attached to the chiral center. The ligands are given a priority (the higher the atomic number the higher the priority) and if the priorities increase in a clockwise direction, they are said to be R-. Otherwise, if they are prioritized in a counterclockwise direction they are said to be S-.

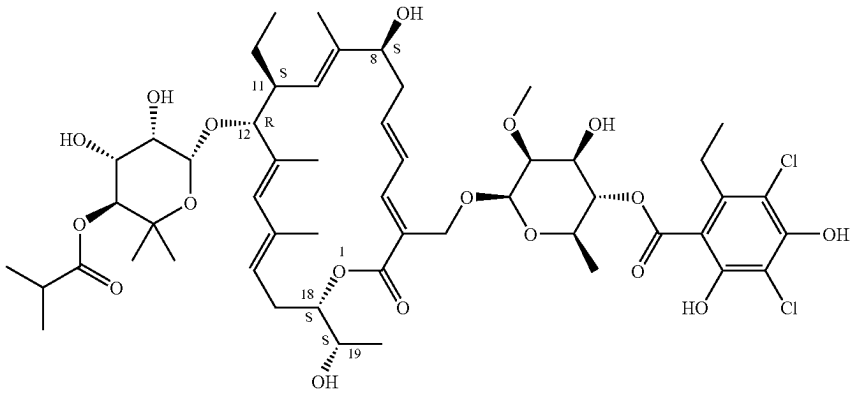
The term "R-Tiacumicin B" refers to the optically pure (R)-isomer of Tiacumicin B with an (R)-hydroxy group at C-19, as shown below in Formula IV:

Formula IV



The term "S-Tiacumicin B" refers to the optically pure (S)-isomer of Tiacumicin B with an (S)-hydroxy group at C-19, as shown below in Formula V:

Formula V



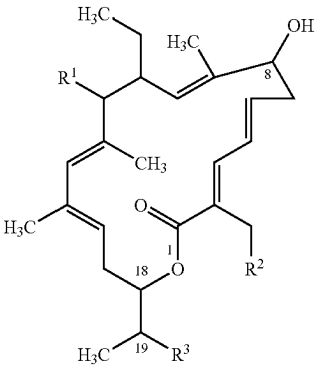
The term "stereoisomers" refers to compounds whose molecules have the same number and kind of atoms and the same atomic arrangement, but differ in their spatial arrangement.

As used herein, and unless otherwise indicated, the terms "optically pure," "stereomerically pure," and "substantially stereomerically pure" are used interchangeably and mean one stereoisomer of a compound or a composition that comprises one stereoisomer of a compound and is substantially free of other stereoisomer(s) of that compound. For example, a stereomerically pure compound or composition of a compound having one chiral center will be substantially free of the opposite enantiomer of the compound. A stereomerically pure compound or composition of a compound having two chiral centers will be substantially free of other diastereomers of the compound. A typical stereomerically pure compound comprises greater than about 80% by weight of one stereoisomer of the compound and less than about 20% by weight of other stereoisomers of the compound, more preferably greater than about 90% by weight of one stereoisomer of the compound and less than about 10% by weight of the other stereoisomers of the compound, even more preferably greater than about 95% by weight of one stereoisomer of the compound and less than about 5% by weight of the other stereoisomers of the compound, and most preferably greater than about 97% by weight of one stereoisomer of the compound and less than about 3% by weight of the other stereoisomers of the compound.

The term "sugar" generally refers to mono-, di- or oligosaccharides. A saccharide may be substituted, for example, glucosamine, galactosamine, acetylglucose, acetylgalactose, N-acetylglucosamine, N-acetyl-galactosamine, galactosyl-N-acetylglucosamine, N-acetylneuraminic acid (sialic acid), etc., as well as sulfated and phosphorylated sugars. For the purposes of this definition, the saccharides are in their pyranose or furanose form.

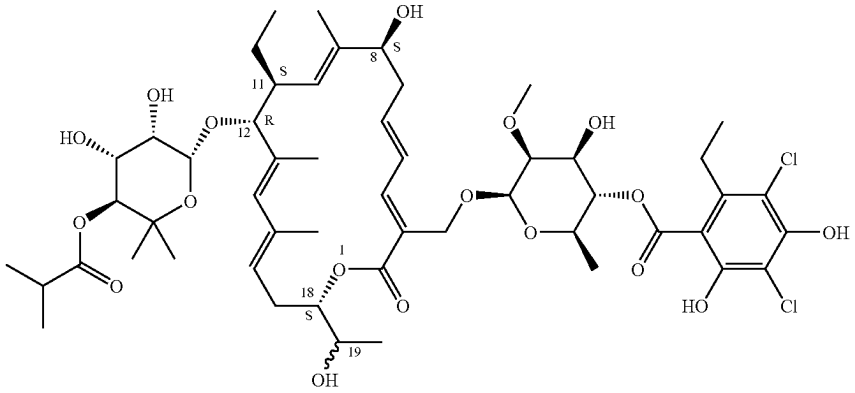
The term "Tiacumicin" as used herein refers to a family of compounds all of which comprise the 18-membered macrocycle shown below in Formula I:

Formula I



The term "Tiacumicin B" as used herein refers to the 18-membered macrocycle shown below in Formula VI:

Formula VI



The term "yield" as used herein refers to an amount of crude Tiacumicin re-constituted in methanol to the same volume as the original fermentation broth. Yield is determined using standard HPLC techniques. Yield is reported in units of mg/L.

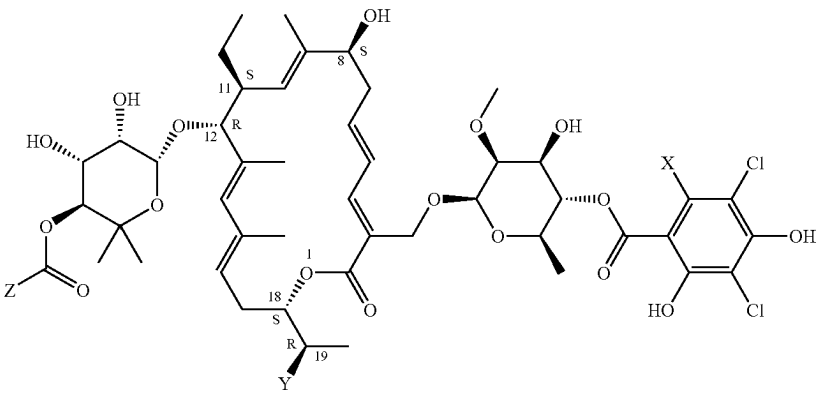
This invention encompasses the composition of novel antibiotic agents, Tiacumicins, by submerged aerobic fermentation of the microorganism *Dactylosporangium aurantiacum* subspecies *hamdenensis*. The production method is covered by WO 2004/014295 A2.

The present invention relates to new antibacterial compositions containing R-Tiacumicins, specifically the R-Tiacumicin B (which has an R-hydroxyl at C-19), and to the use of these new compositions in combination with existing drugs to treat infections caused by gram-positive anaerobes.

The present invention further relates to stereoisomerically pure Tiacumicin B, which contains 90-100% of the R-stereoisomer, preferably at least 93% of the R-stereoisomer, more preferably 95% of the R-stereoisomer, even more preferably 99% of the R-stereoisomer.

In accordance with the present invention there are provided compounds with the structure of Formula VII:

Formula VII



wherein:

X is selected from lower alkyl, and wherein the term "lower alkyl" as used herein refers to branched or straight chain alkyl groups comprising one to two carbon atoms, including methyl, ethyl, n-propyl, isopropyl, and the like; and

Y is selected from OH or a ketone (=O); and

Z is selected from H or lower alkyl, and wherein the term "lower alkyl" as used herein refers to branched or straight chain alkyl groups comprising one to five carbon atoms, including methyl, ethyl, propyl, isopropyl, n-butyl, t-butyl, and the like.

Preferred compounds of the invention are compounds of Formula VII wherein X is methyl or ethyl, Y is ketone (=O) or OH and Z is isopropyl.

More preferred compounds of the invention are the compound of the Formula VII wherein X is ethyl, Y is ketone (=O) or OH and Z is isopropyl.

The most preferred compounds of the invention are the compounds of Formula VII wherein X is ethyl, Y is OH R and Z is isopropyl.

One embodiment of the present invention is directed towards the discovery that the chiral center at C-19 of Tiacumicin B has great effect on biological activity. It has now been discovered that R-Tiacumicin B, which has an R-hydroxy group at C-19 has significantly higher activity than the S-Tiacumicin B and other Tiacumicin B related compounds (Lipiarmycin A4 and C-19 Ketone). The higher activity is shown by much lowered MIC values, which can be seen below in Example 3, Tables 3 and 4 for several strains of *C. difficile, S. aureus, E. faecalis*, and *E. faecium*. This effect of the C-19 chiral center on biological activity is an unexpected and novel discovery.

In another embodiment of the present invention OPT-80 (which is composed almost entirely of the R-Tiacumicin B) has an unusually long post-antibiotic effect (PAE). This is discussed below in Example 4, where it is shown that OPT-80 has a PAE of greater than 24 hours. This PAE is unexpectedly longer than the usual antibiotic PAE of 1-5 hours.

The present invention also relates to the disclosure of pharmaceutical compositions, which comprise a compound of the present invention in combination with a pharmaceutically acceptable carrier.

Yet another aspect of the invention discloses a method of inhibiting or treating bacterial infections in humans, comprising administering to the patient a therapeutically effective amount of a compound of the invention alone or in combination with another antibacterial or antifungal agent.

Production

The 18-membered macrocycles and analogs thereof are produced by fermentation. Cultivation of *Dactylosporangium aurantiacum* subsp. *hamdenensis* AB 718C-41 NRRL 18085 for the production of the Tiacumicins is carried out in a medium containing carbon sources, inorganic salts and other organic ingredients with one or more absorbents under proper aeration conditions and mixing in a sterile environment.

The microorganism to produce the active antibacterial agents was identified as belonging to the family *Actinoplanaceae*, genus *Dactylosporangium* (*J. of Antibiotics,* 1987, 40: 567-574 and U.S. Pat. No. 4,918,174). It has been designated *Dactylasporangium aurantiacum* subspecies *hamdenensis* 718C-41. The subculture was obtained from the ARS Patent Collection of the Northern Regional Research Center, United States Department of Agriculture, 1815 North University Street, Peoria, Ill. 61604, U.S.A., where it was assigned accession number NRRL 18085. The characteristics of strain AB 718C-41 are given in the Journal of Antibiotics, 1987, 40: 567-574 and U.S. Pat. No. 4,918,174.

Methods of isolating stereomerically pure isomers are known in the art. Methods of isolating stereomerically pure R-Tiacumicin include, but are not limited to, recrystallization of the crude mixture in solvents including, aqueous methanol or isopropanol and chiral HPLC.

This invention encompasses the composition of novel antibiotic agents, Tiacumicins, by submerged aerobic fermentation of the microorganism *Dactylosporangium aurantiacum* subspecies *hamdenensis*. The production method is covered by WO 2004/014295 A2, which is hereby incorporated by reference.

Pharmaceutical Formulation and Administration

Pharmaceutical compositions of the Tiacumicin compounds of the present invention, specifically OPT-80 (which is composed almost entirely of the R-Tiacumicin), according to the invention may be formulated to release an antibiotic substantially immediately upon administration or at any predetermined time or time period after administration.

The latter types of compositions are generally known as modified release formulations, which include formulations that create a substantially constant concentration of the drug within the intestinal tract over an extended period of time, and formulations that have modified release characteristics based on temporal or environmental criteria as described in Modified-Release Drug Delivery Technology, ed. M. J. Rathbone, J. Hodgraft and M. S. Roberts. Marcel Dekker, Inc. New York.

Any oral biologically-acceptable dosage form, or combinations thereof, can be employed in the methods of the invention. Examples of such dosage forms include, without limitation, chewable tablets, quick dissolve tablets, effervescent tablets, reconstitutable powders, elixirs, liquids, suppositories, creams, solutions, suspensions, emulsions, tablets, multi-layer tablets, bi-layer tablets, capsules, soft gelatin capsules, hard gelatin capsules, osmotic tablets, osmotic capsules, caplets, lozenges, chewable lozenges, beads, powders, granules, particles, microparticles, dispersible granules, ingestibles, infusions, health bars, confections, animal feeds, cereals, cereal coatings, foods, nutritive foods, functional foods and combinations thereof. The preparation of any of the above dosage forms is well known to persons of ordinary skill in the art. Additionally, the pharmaceutical formulations may be designed to provide either immediate or controlled release of the antibiotic upon reaching the target site. The selection of immediate or controlled release compositions depends upon a variety of factors including the species and antibiotic susceptibility of Gram-positive bacteria being treated and the bacteriostatic/bactericidal characteristics of the therapeutics. Methods well known in the art for making formulations are found, for example, in Remington: The Science and Practice of Pharmacy (20th ed.), ed. A. R. Gennaro, 2000, Lippincott Williams & Wilkins, Philadelphia, or in Encyclopedia of Pharmaceutical Technology, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York.

Immediate release formulations for oral use include tablets or capsules containing the active ingredient(s) in a mixture with non-toxic pharmaceutically acceptable excipients. These excipients may be, for example, inert diluents or fillers (e.g., sucrose, sorbitol, sugar, mannitol, microcrystalline cellulose, starches including potato starch, calcium carbonate, sodium chloride, lactose, calcium phosphate, calcium sulfate, or sodium phosphate); granulating and disintegrating agents (e.g., cellulose derivatives including microcrystalline cellulose, starches including potato starch, croscarmellose sodium, alginates, or alginic acid); binding agents (e.g., sucrose, glucose, mannitol, sorbitol, acacia, alginic acid, sodium alginate, gelatin, starch, pregelatinized starch, microcrystalline cellulose, magnesium aluminum silicate, carboxymethylcellulose sodium, methylcellulose, hydroxypropyl methylcellulose, ethylcellulose, polyvinylpyrrolidone, or polyethylene glycol); and lubricating agents, glidants, and antiadhesives (e.g., magnesium stearate, zinc stearate, stearic acid, silicas, hydrogenated vegetable oils, or talc). Other pharmaceutically acceptable excipients can be colorants, flavoring agents, plasticizers, humectants, buffering agents, and the like as are found, for example, in The Handbook of Pharmaceutical Excipients, third edition, edited by Arthur H. Kibbe, American Pharmaceutical Association Washington D.C.

Dissolution or diffusion controlled release can be achieved by appropriate coating of a tablet, capsule, pellet, or granulate formulation of compounds, or by incorporating the compound into an appropriate matrix. A controlled release coating may include one or more of the coating substances mentioned above and/or, e.g., shellac, beeswax, glycowax, castor wax, carnauba wax, stearyl alcohol, glyceryl monostearate, glyceryl distearate, glycerol palmitostearate, ethylcellulose, acrylic resins, dl-polylactic acid, cellulose acetate butyrate, polyvinyl chloride, polyvinyl acetate, vinyl pyrrolidone, polyethylene, polymethacrylate, methylmethacrylate, 2-hydroxymethacrylate, methacrylate hydrogels, 1,3 butylene glycol, ethylene glycol methacrylate, and/or polyethylene glycols. In a controlled release matrix formulation, the matrix material may also include, e.g., hydrated methylcellulose, carnauba wax and stearyl alcohol, carbopol 934, silicone, glyceryl tristearate, methyl acrylate-methyl methacrylate, polyvinyl chloride, polyethylene, and/or halogenated fluorocarbon.

A controlled release composition may also be in the form of a buoyant tablet or capsule (i.e., a tablet or capsule that, upon oral administration, floats on top of the gastric content for a certain period of time). A buoyant tablet formulation of the compound(s) can be prepared by granulating a mixture of the antibiotic with excipients and 20-75% W/W of hydrocolloids, such as hydroxyethylcellulose, hydroxypropylcellulose, or hydroxypropyl-methylcellulose. The obtained granules can then be compressed into tablets. On contact with the gastric juice, the tablet forms a substantially water-impermeable gel barrier around its surface. This gel barrier takes part in maintaining a density of less than one, thereby allowing the tablet to remain buoyant in the gastric juice. Other useful controlled release compositions are known in the art (see, for example, U.S. Pat. Nos. 4,946,685 and 6,261,601).

A modified release composition may be comprised of a compression-coated core whose geometric configuration controls the release profile of the encapsulated antibiotic. By varying the geometry of the core, the profile of the antibiotic release can be adjusted to follow zero order, first order or a combination of these orders. The system can also be designed to deliver more beneficial agents at the same time, each having a different release profile (see, for example U.S. Pat. Nos. 4,111,202 and 3,279,995).

Formulations that target the Tiacumicin compounds of the present invention, specifically OPT-80 (which is composed almost entirely of the R-Tiacumicin), that release to particular regions of the intestinal tract can also be prepared. The Tiacumicin compounds of the present invention, specifically OPT-80, can be encapsulated in an enteric coating that prevents release degradation and release from occurring in the stomach, but dissolves readily in the mildly acidic or neutral pH environment of the small intestine. A formulation targeted for release of antibiotic to the colon, utilizing technologies such as time-dependent, pH-dependent, or enzymatic erosion of polymer matrix or coating can also be used.

The targeted delivery properties of the Tiacumicin compounds of the present invention, specifically OPT-80 (which is composed almost entirely of the R-Tiacumicin B), containing formulation may be modified by other means. For example, the antibiotic may be complexed by inclusion, ionic association, hydrogen bonding, hydrophobic bonding, or

covalent bonding. In addition polymers or complexes susceptible to enzymatic or microbial lysis may also be used as a means to deliver drug.

Microsphere encapsulation of the Tiacumicin compounds of the present invention, specifically OPT-80 (which is composed almost entirely of the R-Tiacumicin B), is another useful pharmaceutical formulation for targeted antibiotic release. The antibiotic-containing microspheres can be used alone for antibiotic delivery, or as one component of a two-stage release formulation. Suitable staged release formulations may consist of acid stable microspheres, encapsulating the compounds of the present invention, specifically OPT-80 (which is composed almost entirely of the R-Tiacumicin B), to be released later in the lower intestinal tract admixed with an immediate release formulation to deliver antibiotic to the stomach and upper duodenum.

Microspheres can be made by any appropriate method, or from any pharmaceutically acceptable material. Particularly useful are proteinoid microspheres (see, for example, U.S. Pat. Nos. 5,601,846, or 5,792,451) and PLGA-containing microspheres (see, for example, U.S. Pat. Nos. 6,235,224 or 5,672,659). Other polymers commonly used in the formation of microspheres include, for example, poly-ϵ-caprolactone, poly(e~caprolactone-Co-DL-lactic acid), poly(DL-lactic acid), poly(DL-lactic acid-Co-glycolic acid) and poly(s-caprolactone-Co-glycolic acid) (see, for example, Pitt et al., J. Pharm. Sci., 68:1534, 1979). Microspheres can be made by procedures well known in the art including spray drying, coacervation, and emulsification (see for example Davis et al. Microsphere and Drug Therapy, 1984, Elsevier; Benoit et al. Biodegradable Microspheres: Advances in Production Technologies, Chapter 3, ed. Benita, S, 1996, Dekker, New York; Microencapsulation and Related Drug Processes, Ed. Deasy, 1984, Dekker, New York; U.S. Pat. No. 6,365,187).

Powders, dispersible powders, or granules suitable for preparation of aqueous solutions or suspensions of the Tiacumicin compounds of the present invention, specifically OPT-80 (which is composed almost entirely of the R-Tiacumicin B), by addition of water are convenient dosage forms for oral administration. Formulation as a suspension provides the active ingredient in a mixture with a dispersing or wetting agent, suspending agent, and one or more preservatives. Suitable dispersing or wetting agents are, for example, naturally-occurring phosphatides (e.g., lecithin or condensation products of ethylene oxide with a fatty acid, a long chain aliphatic alcohol, or a partial ester derived from fatty acids) and a hexitol or a hexitol anhydride (e.g., polyoxyethylene stearate, polyoxyethylene sorbitol monooleate, polyoxyethylene sorbitan monooleate, and the like). Suitable suspending agents are, for example, sodium carboxymethylcellulose, methylcellulose, sodium alginate, and the like.

EXAMPLES

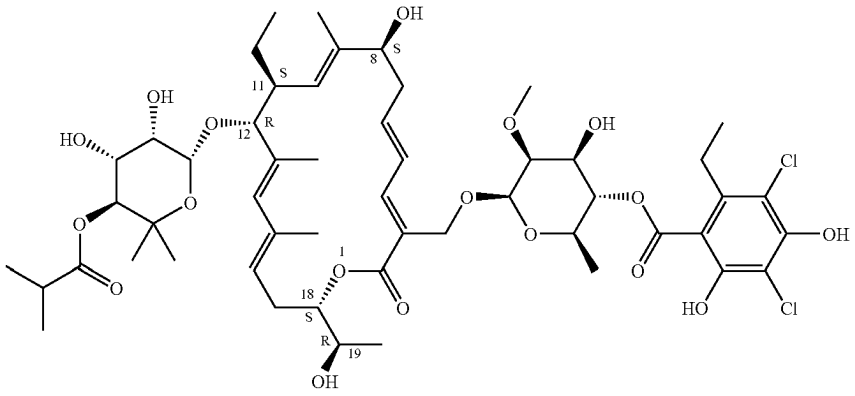
The following examples are provided by way of describing specific embodiments of the present invention without intending to limit the scope of the invention in any way.

Example 1

Exact Structure of R-Tiacumicin B

The exact structure of the R-Tiacumicin B (the major most active component of OPT-80) is shown below in Formula IV. The X-ray crystal structure of the R-Tiacumicin B was obtained from a colorless, parallelepiped-shaped crystal (0.08×0.14×0.22 mm) grown in methanol and is shown as an ORTEP diagram in FIG. **1**. This x-ray structure confirms the structure shown below in Formula IV. The official chemical name is 3-[[[6-Deoxy-4-O-(3,5-dichloro-2-ethyl-4,6-dihydroxybenzoyl)-2-O-methyl-β-D-mannopyranosyl]oxy]-methyl]-12(R)-[[6-deoxy-5-C-methyl-4-O-(2-methyl-1-oxopropyl)-β-D-lyxo-hexopyranosyl]oxy]-11(S)-ethyl-8(S)-hydroxy-18(S)-(1(R)-hydroxyethyl)-9,13,15-trimethyloxacyclooctadeca-3,5,9,13,15-pentaene-2-one.

Formula IV



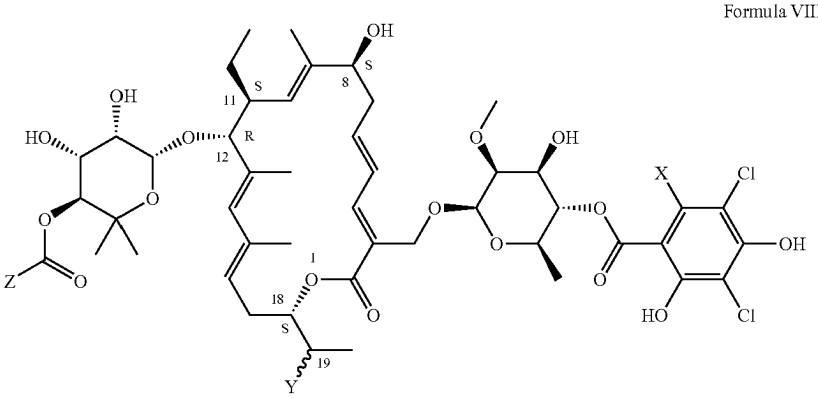
Example 2

Analytical Data of OPT-80 and Related Substances

The analytical data of OPT-80 (which is composed almost entirely of the R-Tiacumicin B, which is the most active component of OPT-80) and three related compounds (S-Tiacumicin B, Lipiarmycin A4, and C-19 ketone) are summarized below. The structures of these compounds are shown in Formula VIII and Table 2 below.

TABLE 2

Formula VIII



Structure of R-Tiacumicin B (the major most active component of OPT-80) and related substances

Compound	X	Y	Z
R-Tiacumicin B	Ethyl	(R)—OH	Isopropyl
S-Tiacumicin B	Ethyl	(S)—OH	Isopropyl
Lipiarmycin A4	Methyl	(S)—OH	Isopropyl
C-19 Ketone	Ethyl	=O	Isopropyl

Analytical Data of R-Tiacumicin B

mp 166-169° C. (white needle from isopropanol);

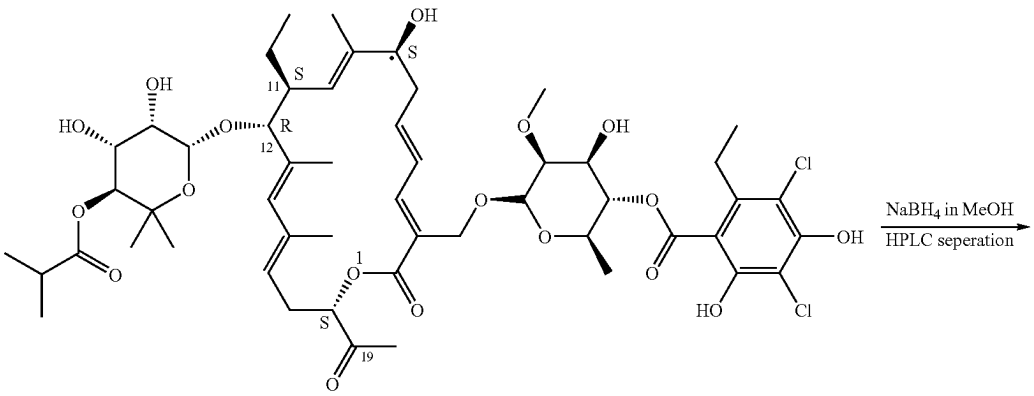
$[\alpha]_D^{20}$ −6.9 (c 2.0, MeOH);

MS m/z (ESI) 1079.7 $(M+Na)^+$;

^1H ^1H NMR NMR (400 MHz, CD_3OD) δ 7.21 (d, 1H), 6.59 (dd, 1H), 5.95 (ddd, 1H), 5.83 (br s, 1H), 5.57 (t, 1H), 5.13 (br d, 1H), 5.09 (t, 1H), 5.02 (d, 1H), 4.71 (m, 1H), 4.71 (br s, 1H), 4.64 (br s, 1H), 4.61 (d, 1H), 4.42 (d, 1H), 4.23 (m, 1H), 4.02 (pentet, 1H), 3.92 (dd, 1H), 3.73 (m, 2H), 3.70 (d, 1H), 3.56 (s, 3H), 3.52-3.56 (m, 2H), 2.92 (m, 2H), 2.64-2.76 (m, 3H), 2.59 (heptet, 1H), 2.49 (ddd, 1H), 2.42 (ddd, 1H), 2.01 (dq, 1H), 1.81 (s, 3H), 1.76 (s, 3H), 1.65 (s, 3H), 1.35 (d, 3H), 1.29 (m, 1H), 1.20 (t, 3H), 1.19 (d, 3 H), 1.17 (d, 3H), 1.16 (d, 3H), 1.14 (s, 3H), 1.12 (s, 3H), 0.87 (t, 3H);

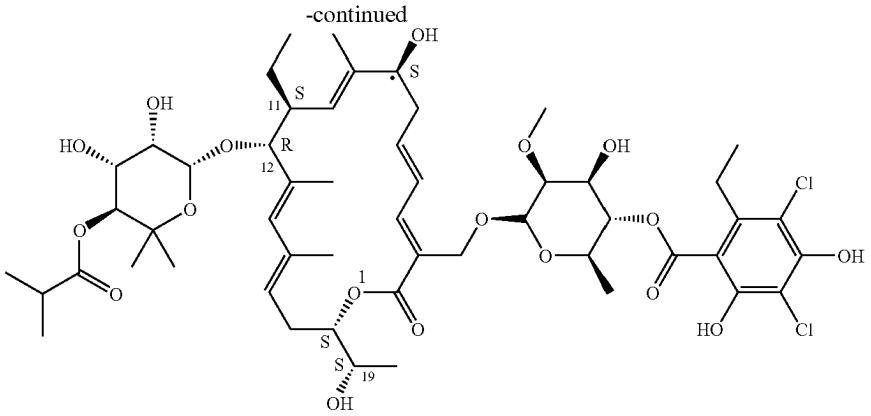
^{13}C NMR (100 MHz, CD_3OD) δ 178.4, 169.7, 169.1, 154.6, 153.9, 146.2, 143.7, 141.9, 137.1, 137.0, 136.4, 134.6, 128.5, 126.9, 125.6, 124.6, 114.8, 112.8, 108.8, 102.3, 97.2, 94.3, 82.5, 78.6, 76.9, 75.9, 74.5, 73.5, 73.2, 72.8, 71.6, 70.5, 68.3, 63.9, 62.2, 42.5, 37.3, 35.4, 28.7, 28.3, 26.9, 26.4, 20.3, 19.6, 19.2, 18.7, 18.2, 17.6, 15.5, 14.6, 14.0, 11.4.

Analytical Data of the S-Tiacumicin B



Formula II (C-19 Ketone)

-continued



Formula V (S-Tiacumicin B)

$NaBH_4$ (9 eq, 48 mg) was added in three portions to a solution of C-19 Ketone (150 mg) in 3 mL MeOH. After 1 h, saturated NH_4Cl solution was added. The mixture was extracted with $CHCl_3$, and then concentrated. S-Tiacumicin B was purified by YMC-pack ODS-A 75×30 mm I.D. column (H_2O:MeOH:AcOH 28:72:1) yielding pure 35 mg of pure S-Tiacumicin B.

MS m/z 1074.5 $(M+NH_4)^+$;

^1H NMR (400 MHz, $CDCl_3$) δ 7.15 (d, J=11.4 Hz, 1H), 6.58 (dd, J=14.1, 11.4 Hz, 1H), 5.82 (ddd, J=14.1, 10.6, 3.5 Hz, 1H), 5.78 (s, 1H), 5.40 (dd, J=7.8, 7.8 Hz, 1H), 5.15 (dd, J=9.5, 9.5 Hz, 1H), 5.01 (d, J=9.9 Hz, 1H), 5.01 (d, J=9.9 Hz, 1H), 4.77 (ddd, J=5.8, 5.3, 5.3 Hz, 1H), 4.68 (d, J=11.6 Hz, 1H), 4.65 (br s, 1H), 4.62 (br s, 1H), 4.42 (d, J=11.6 Hz, 1H), 4.28 (br s, 1H), 4.07-3.97 (m, 2H), 3.74-3.58 (m, 4H), 3.61 (s, 3H), 3.52 (dq, J=9.5, 5.8 Hz, 1H), 3.08 (dq, J=12.6, 6.1 Hz, 1H), 3.01 (dq, J=12.6, 6.1 Hz, 1H), 2.77-2.65 (m, 2H), 2.60 (heptet, J=6.9 Hz, 1H), 2.55-2.44 (m, 3H), 1.95-1.84 (m, 1H), 1.80 (s, 3H), 1.76 (s, 3H), 1.66 (s, 3H), 1.34 (d, J=5.8 Hz, 3H), 1.29-1.24 (m, 1H), 1.27 (d, J=6.6 Hz, 3H), 1.21 (t, J=6.1 Hz, 3H), 1.19 (d, J=6.9 Hz, 3H), 1.18 (d, J=6.9 Hz, 3H), 1.15 (s, 3H), 1.10 (s, 3H), 0.84 (t, J=7.2 Hz, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 177.4, 170.1, 168.8, 157.6, 152.8, 144.4, 143.1, 141.1, 136.7, 136.2, 134.9, 133.8, 128.7, 125.7, 125.2, 123.0, 113.9, 107.5, 107.2, 101.7, 94.9, 92.6, 80.8, 79.2, 76.6, 74.8, 73.5, 72.7, 71.9, 71.7, 70.2, 70.1, 69.5, 63.5, 62.3, 41.5, 36.6, 34.3, 29.5, 28.2, 26.2, 26.0, 19.4, 19.3, 18.9, 18.5, 17.8, 17.3, 15.3, 14.1, 13.7, 11.1;

Analytical Data of Lipiarmycin A_4

MS m/z 1060.5 $(M+NH_4)^+$;

^1H NMR (400 MHz, $CDCl_3$) δ 7.12 (d, J=11.6 Hz, 1H), 6.59 (dd, J=14.1, 11.6 Hz, 1H), 5.85 (br s, 1H), 5.83 (ddd, J=14.1, 10.6, 4.8 Hz, 1H), 5.47 (dd, J=8.3, 8.3 Hz, 1H), 5.12 (dd, J=9.6, 9.6 Hz, 1H), 5.00 (d, J=10.1 Hz, 1H), 4.98 (br d, J=10.6 Hz, 1H), 4.75-4.69 (m, 1H), 4.68 (d, J=11.4 Hz, 1H), 4.66 (br s, 1H), 4.62 (br s, 1H), 4.40 (d, J=11.4 Hz, 1H), 4.26 (br s, 1H), 4.07-4.00 (m, 1H), 4.02 (br d, J=3.3 Hz, 1H), 3.75-3.61 (m, 4H), 3.62 (s, 3H), 3.55 (dq, J=9.6, 6.1 Hz, 1H), 2.82-2.45 (m, 6H), 2.60 (s, 3H), 2.07-1.97 (m, 1H), 1.92 (s, 3H), 1.81 (s, 3H), 1.67 (s, 3H), 1.32 (d, J=6.1 Hz, 3H), 1.30-1.22 (m, 1H), 1.21 (d, J=6.6 Hz, 3H), 1.19 (d, J=7.1 Hz, 3H), 1.18 (d, J=7.1 Hz, 3H), 1.15 (s, 3H), 1.10 (s, 3H), 0.83 (t, J=7.2 Hz, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 177.4, 170.5, 168.9, 157.8, 153.0, 144.3, 140.9, 137.7, 137.0, 136.3, 134.6, 134.4, 129.1, 127.9, 125.3, 123.2, 114.5, 107.4, 107.0, 101.8, 94.7, 92.5, 80.3, 79.6, 76.7, 74.9, 73.5, 72.7, 71.9, 71.6, 70.2, 70.1, 69.1, 63.6, 62.3, 41.9, 36.9, 34.4, 28.8, 28.2, 25.9, 20.0, 19.3, 19.0, 18.6, 18.5, 17.8, 17.2, 15.5, 13.8, 11.2;

Analytical Data of C-19 Ketone

MS m/z 1072.5 $(M+NH_4)^+$;

^1H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, J=11.4 Hz, 1H), 6.61 (dd, J=14.7, 11.4 Hz, 1H), 5.91 (ddd, J=14.7, 9.1, 5.8 Hz, 1H), 5.83 (s, 1H), 5.31 (dd, J=7.9, 7.9 Hz, 1H), 5.14 (dd, J=9.7, 9.7 Hz, 1H), 5.06 (d, J=10.6 Hz, 1H), 5.00 (d, J=10.1 Hz, 1H), 4.98 (dd, J=7.1, 4.8 Hz, 1H), 4.67 (d, J=11.9 Hz, 1H), 4.66 (br s, 1H), 4.61 (br s, 1H), 4.42 (d, J=11.9 Hz, 1H), 4.30 (br s, 1H), 4.02 (br d, J=3.3 Hz, 1H), 3.63-3.60 (m, 4H), 3.62 (s, 3H), 3.51 (dq, J=9.7, 6.1 Hz, 1H), 3.09 (dq, J=14.4, 7.3 Hz, 1H), 3.03 (dq, J=14.4, 7.3 Hz, 1H), 2.76-2.50 (m, 6H), 2.21 (s, 3H), 1.93-1.87 (m, 1H), 1.87 (s, 3H), 1.75 (s, 3H), 1.63 (s, 3H), 1.32 (d, J=6.1 Hz, 3H), 1.27-1.22 (m, 1H), 1.21 (t, J=7.3 Hz, 3H), 1.19 (d, J=7.1 Hz, 3H), 1.18 (d, J=7.1 Hz, 3H), 1.14 (s, 3H), 1.10 (s, 3H), 0.84 (t, J=7.3 Hz, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 205.5, 177.4, 170.1, 166.9, 157.6, 152.8, 145.7, 143.1, 142.0, 137.1, 136.8, 135.5, 133.7, 128.3, 124.8, 124.0, 122.8, 113.9, 107.3, 107.2, 101.3, 94.8, 92.4, 80.4, 77.7, 76.6, 74.7, 73.5, 72.6, 71.8, 71.7, 70.2, 70.0, 63.0, 62.3, 41.5, 36.5, 34.3, 29.6, 28.1, 26.2, 26.1, 26.0, 19.2, 18.9, 18.5, 17.8, 17.3, 15.2, 14.0, 13.3, 11.0

Example 3

Biological Activity

MIC Values Determined for Several *C. difficile* Strains

OPT-80 (which is composed almost entirely of the R-Tiacumicin B) and its related compounds were tested against *C. difficile*. The MIC values are reported below in Table 3. OPT-80 was surprisingly active when compared to its enantiomer S-Tiacumicin B and Lipiarmycin A4.

TABLE 3

MIC (μg/ml) versus *C. difficile* strains

C. difficile strains	R-Tiacumicin B (>90% Stereomerically Pure)	S-Tiacumicin B	Lipiarmycin A4	C-19 Ketone
ATCC 9689	0.03	0.125	0.06	0.06
ATCC 43255	0.125	1	0.5	0.5

TABLE 3-continued

MIC (μg/ml) versus *C. difficile* strains

C. difficile strains	R-Tiacumicin B (>90% Stereomerically Pure)	S-Tiacumicin B	Lipiarmycin A4	C-19 Ketone
ATCC 17857	0.03	0.25	0.06	nd
LC # 1 (Clinical isolate)	0.125	1	0.5	0.5

MIC Values Determined for Various Microorganisms

OPT-80 (which is composed almost entirely of the R-Tiacumicin B) and its related compounds were tested against several other pathogens. The MIC values are reported below in Table 4. OPT-80 was suprisingly active when compared to S-Tiacumicin B and Lipiarmycin A4.

TABLE 4

MIC (μg/ml) against other microorganisms

Strain ID #	Organism	R-Tiacumicin B (>90% Stereomerically Pure)	S-Tiacumicin B	Lipiarmycin A4
1	*S. aureus* (ATCC 29213)	4	64	8
2	*S. aureus*, (MRSA)	4	64	16
3	*S. aureus*, (MRSA)	4	64	8
4	*E. faecalis* (ATCC 29212)	2	8	2
5	*E. faecalis* Vanc. resistant	4	32	16
6	*E. faecalis* Vanc. resistant	1	16	4
7	*E. faecium* Vanc. resistant	1	8	4
8	*E. faecium* Vanc. resistant	1	32	32

Example 4

Post-Antibiotic Effect of OPT-80 in *C. difficile*

The post-antibiotic effect (PAE) of OPT-80 (which is composed almost entirely of the R-Tiacumicin B) was measured versus two strains of *C. difficile*, ATCC 43255 and a clinical isolate, LC3. Vancomycin and rifampin were tested additionally versus LC3.

The PAE at 4× the MIC was observed to be extremely long: greater than 24 hours, for both strains. Because of the long duration of this effect, an exact PAE was not calculated. Vancomycin, on the other hand, had a more normal PAE of less than an hour when used at 4× the MIC versus strain LC3.

Example 5

In Vitro Activity of OPT-80

The in vitro efficacy of OPT-80 (which is composed almost entirely of the R-Tiacumicin B), metronidazole, and vancomycin were assessed versus 110 genetically distinct clinical isolates of *C. difficile* via agar dilution. The MIC data are presented in Tables 5 and 6.

TABLE 5

Geometric mean, MIC ranges, MIC_{50}, and MIC_{90} values for OPT-80against 110 *C. difficile* clinical isolates, vancomycin, and metronidazole, in μg/mL.

	Range	Geometric Mean	MIC_{50}	MIC_{90}
OPT-80	0.015-0.25	0.08	0.125	0.125
Metronidazole	0.025-0.5	0.15	0.125	0.25
Vancomycin	0.06-4	0.8	1	1

TABLE 6

Raw MIC data for OPT-80, vancomycin (VAN), and metronidazole (MTZ) versus 110 clinical isolates of *C. difficile*, in μg/mL.

ORG ID	R-Tiacumicin B (>90% Stereomerically Pure)	MTZ	VAN
A1 1535	0.125	0.25	1
B1 832	0.06	0.125	1
D1 1360	0.03	0.25	1
E1 816	0.06	0.125	1
F1 1015	0.125	0.125	1
G1 1077	0.125	0.125	1
I1 1389	0.125	0.125	1
J1 5971	0.06	0.25	1
J7 4224	0.03	0.125	1
J9 4478	0.06	0.125	1
K1 4305	0.125	0.25	0.5
K14 5780	0.125	0.125	1
L1 1423	0.125	0.125	0.5
N1 471	0.125	0.125	0.5
O1 1861	0.06	0.125	1
R1 397	0.125	0.125	1
R6 6015	0.015	0.25	2
V1 1521	0.125	0.125	0.5
W1 3931	0.125	0.5	1
X1 1890	0.125	0.125	1
Y1 5639	0.06	0.125	0.5
Y2 1459	0.06	0.125	1
Z1 3036	0.03	0.125	1
AA2 4380	0.015	0.125	1
AB2 1725	0.06	0.125	1
AC1 1546	0.06	0.125	1
AF1 1808	0.125	0.125	0.5
AG1 3044	0.125	0.125	1
AH1 3430	0.125	0.25	0.5
AJ1 1557	0.06	0.125	1
AL1 1753	0.06	0.125	0.5
AN1 464	0.125	0.125	0.5
AO1 287	0.125	0.125	1
AS1 4099	0.125	0.125	1
AT1 1216	0.125	0.125	1
AV1 941	0.25	0.125	0.5
CJ1 893	0.125	0.025	1
AW1 4501	0.125	0.125	1
BE1 4307	0.125	0.25	1
BH1 4506	0.06	0.06	0.5
BI1 1675	0.125	0.125	1
BK1 4291	0.125	0.125	0.5
BL1 716	0.125	0.125	1
BM1 1453	0.06	0.125	1
BN1 1322	0.125	0.25	1
BR1 1321	0.06	0.125	1
BT1 706	0.06	0.125	1
BV1 1183	0.125	0.25	1
BW1 3130	0.125	0.125	1
BX1 4271	0.125	0.25	1
CN1 667	0.25	0.25	1
CB1 1584	0.25	0.125	1
CF1 5922	0.125	0.125	1
CG1 1566	0.125	0.125	1
CL1 3851	0.25	0.125	1
CO1 4652	0.25	0.125	1
CP1 5491	0.125	0.25	1
61 5930	0.03	0.25	1

TABLE 6-continued

Raw MIC data for OPT-80, vancomycin (VAN), and metronidazole (MTZ) versus 110 clinical isolates of *C. difficile*, in μg/mL.

ORG ID	R-Tiacumicin B (>90% Stereomerically Pure)	MTZ	VAN
63 6029	0.25	0.25	0.06
64 5940	0.125	0.25	1
65 5967	0.06	0.25	0.5
66 6366	0.015	0.125	0.5
67 6367	0.125	0.25	1
68 6368	0.03	0.125	0.06
69 6370	0.25	0.25	0.5
70 6376	0.125	0.25	2
71 6379	0.125	0.25	1
72 6380	0.125	0.25	2
73 6382	0.25	0.25	1
75 6388	0.125	0.125	0.5
76 6389	0.125	0.25	0.5
77 6390	0.06	0.125	1
78 6392	0.015	0.03	0.5
80 6327	0.125	0.125	0.5
81 6328	0.125	0.125	0.5
82 6329	0.06	0.03	0.5
83 6330	0.06	0.125	0.5
84 6331	0.125	0.25	0.5
85 6332	0.06	0.125	1
86 6333	0.03	0.125	0.5
87 6334	0.125	0.125	0.5
88 6335	0.125	0.25	0.5
89 6336	0.25	0.5	1
90 6338	0.125	0.125	1
91 6339	0.125	0.125	1
93 6341	0.125	0.125	1
94 6343	0.015	0.06	0.5
95 6347	0.125	0.125	1
96 6348	0.06	0.125	0.5
97 6349	0.25	0.125	1
98 6350	0.125	0.5	1
101 6354	0.015	0.06	1
102 6355	0.016	0.125	1
103 6068	0.06	0.125	1
104 6060	0.03	0.25	1
105 6071	0.03	0.125	0.5
106 6078	0.03	0.25	0.5
107 6079	0.06	0.125	0.5
109 6274	0.015	0.125	1
111 6279	0.03	0.125	1
112 6280	0.06	0.125	0.5
113 6304	0.06	0.125	1
114 386	0.06	0.125	4
115 5985	0.015	0.25	2
116 5702	0.06	0.125	1
117 6026	0.06	0.125	2
120 6057	0.03	0.25	1
121 6072	0.06	0.25	0.5
122 6111	0.25	0.25	0.5
100 6353	0.125	0.25	1

Example 6

Activity of OPT-80 Compared Against Selected Anaerobic Species

The in vitro activity of OPT-80 was determined against 350 anaerobes. The experimental procedure for which is outlined in Antimicrobial Agents and Chemotherapy, 2004, 48: 4430-4434, which is hereby incorporated by reference in its entirety.

All organisms, including the 21 *C. difficile* strains, were separate isolates and not clonally related. All quality-control gram-negative and -positive strains recommended by NCCLS were included with each run: in every case, results (where available) were in range.

Results of MIC testing are presented in Table 7.

TABLE 7

MICs (μg/ml) of R-Tiacumicin B (>90% Stereomerically Pure)

Organism	MIC range	MIC_{50}	MIC_{90}
Bacteroides fragilis (19)	64–>128	>128	>128
Non-*fragilis B. fragilis* group species (38)	64–>128	>128	>128
Prevotella/Porphyromonas species (42)	16–>128	>128	>128
Fusobacterium nucleatum (14)	64–>128	>128	>128
Fusobacterium mortiferum (10)	64–>128	>128	>128
Fusobacterium species, miscellaneous (14)	16–>128	>128	>128
Peptostreptococcus tetradius (16)	0.25-2.0	1.0	1.0
Peptostreptococcus asaccharolyticus (15)	0.25-1.0	0.5	1.0
Peptostreptococcus anaerobius (15)	<0.016-0.03	<0.016	<0.016
Finegoldia magna (15)	0.25-2.0	1.0	1.0
Micromonas micros (14)	<0.016-0.06	0.03	0.06
Peptostreptococcus prevotii (3)	0.25-1.0	NA	NA
Propionibacterium acnes (20)	0.5-1.0	4.0	4.0
Eggerthella lenta (10)	<0.016-0.06	<0.016	<0.03
Miscellaneous gram-positive non-spore-forming rods (20)	<0.016-16	<0.125	16
Clostridium perfringens (35)	<0.016-0.06	<0.016	0.03
Clostridium difficile (21)	<0.016-0.25	<0.016	0.125
Clostridium tertium (10)	<0.016-0.06	<0.016	0.03
Clostridium species (19)	<0.016-0.06	<0.016	0.03
Clostridium spp. (all) (85)	<0.016-0.06	<0.016	0.06

Example 7

In Vitro Activities of OPT-80 Against Intestinal Bacteria

The in vitro activity of OPT-80 against intestinal bacteria was evaluated. The experimental procedure for which is outlined in Antimicrobial Agents and Chemotherapy, 2004, 48: 4898-4902, which is hereby incorporated by reference in its entirety.

Antimicrobial concentration ranges were selected to encompass or surpass the levels that would be achieved in the gut (to the extent that this information is available), subject to the limitations of solubility of the drugs in the testing medium. The range of concentration of OPT-80 used during testing was 0.03 μg/ml to 1024 μg/ml.

For analysis, the bacteria tested were generally placed into genus, species, or other groups with at least 10 isolates. The ranges and the MICs at which 50 and 90% of isolates were inhibited were determined except for organisms with fewer than 10 strains tested, for which only the ranges are reported (Table 8).

OPT-80 had good activity against most anaerobic gram-positive non-spore-forming rods and anaerobic gram-positive cocci. OPT-80 also showed good activity against enterococci and staphylococci.

TABLE 8

In vitro activity of R-Tiacumicin B (>90% Stereomerically Pure) against 453 bacterial isolates

Organism	MIC range	MIC_{50}	MIC_{90}
Bacteroides fragilis group spp. (50)	256->1024	256	>1024
Veillonella spp. (10)	16-128	32	128
Other anaerobic gram-negative rods (51)	0.06-1024	1024	>1024
All anaerobic gram-negative species (111)	0.06->1024	256	>1024
Clostridium bifermentans (9)	0.06	NA	NA
Clostridium bolteae (7)	1-64	NA	NA
Clostridium clostridioforme (4)	4-128	NA	NA
Clostridium difficile (23)	0.06-2	0.12	0.25
Clostridium glycolicum (9)	0.06-1	NA	NA
Clostridium innocuum (9)	32-128	NA	NA
Clostridium paraputrificum (8)	0.06-8	NA	NA
Clostridium perfringens (14)	0.06	0.062	0.062
Clostridium ramosum (10)	256-512	512	512
Clostridium sordellii (5)	0.06	NA	NA
Other clostridial species (9)	0.06->1024	NA	NA
All *Clostridium* species (107)	0.06->1024	0.062	128
Anaerobic non-spore-forming gram-positive rods (63)	0.06->1024	1	32
Anaerobic gram-positive cocci (49)	0.06->1024	0.5	2
All anaerobic gram-positive species (219)	0.06->1024	0.12	64
Streptococcus, formerly *S. milleri* group (14)	16-64	32	32
Other *Streptococcus* species (9)	16-128	NA	NA
Enterococcus species (21)	2.0-16	8	8
Staphylococcus aureus and *Staphylococcus epidermidis* (19)	0.25-2	0.5	2
Total for all strains (453)	0.06->1024	8	1024

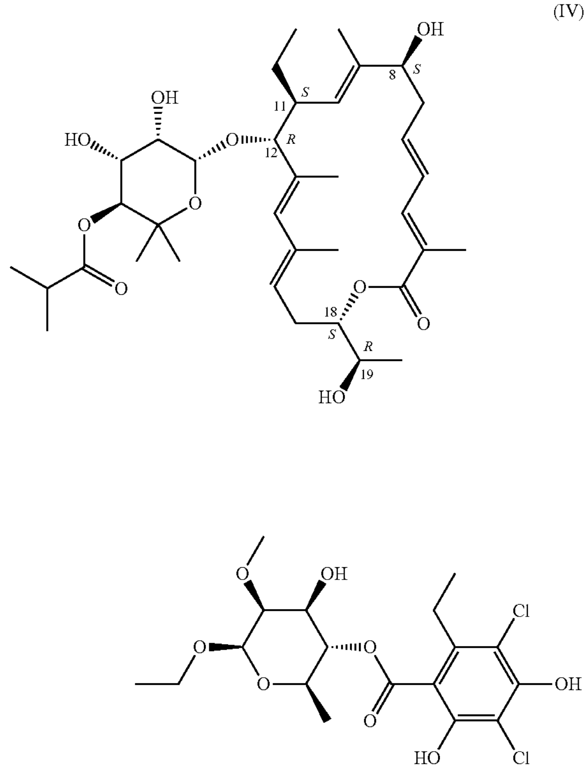
Other Embodiments

All references discussed above are herein incorporated by reference in their entirety for all purposes. While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the appended claims.

What is claimed is:

1. A method of treating diarrhea caused by *C. difficile* gastrointestinal infection in a human patient in need thereof comprising orally administering to said patient a therapeutically effective amount of a compound having the formula (IV):

(IV)



or a pharmaceutically acceptable salt combined with one or more pharmaceutically acceptable carriers, wherein the compound having formula (IV) is greater than 90% by weight stereomerically pure.

2. The method of claim **1**, wherein the compound of formula (IV) is formulated as a tablet.

3. The method of claim **1**, wherein the compound of formula (IV) is formulated as a capsule.

4. The method of claim **1**, wherein the compound of formula (IV) is greater than 93% by weight stereomerically pure.

5. The method of claim **1**, wherein the compound of formula (IV) is greater than 95% by weight stereomerically pure.

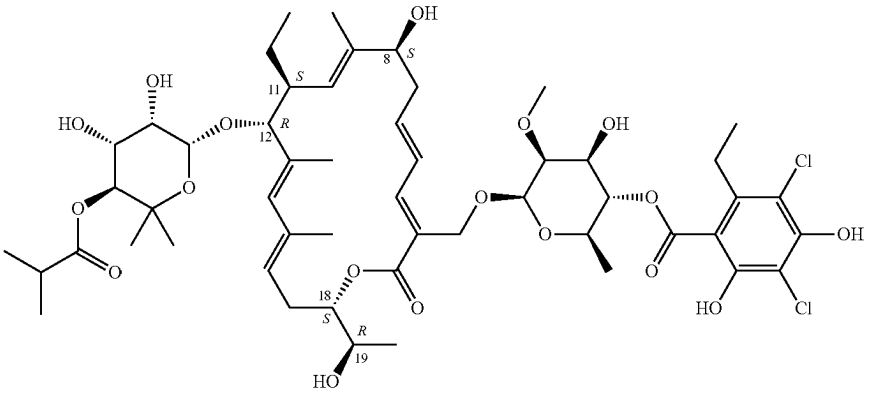
6. The method of claim **1**, wherein the compound of formula (IV) is greater than 97% by weight stereomerically pure.

7. The method of claim **1**, wherein the compound of formula (IV) is substantially free of other diastereomers of the compound.

8. The method of claim **1**, wherein the method consists of administering to the human patient a therapeutically effective amount of the compound having formula (IV) or a pharmaceutically acceptable salt thereof combined with one or more pharmaceutically acceptable carriers.

9. A method of treating diarrhea caused by *C. difficile* gastrointestinal infection in a human patient in need thereof consisting of orally administering to said patient a therapeutically effective amount of a compound having the formula (IV):

(IV)



or a pharmaceutically acceptable salt thereof combined with one or more pharmaceutically acceptable carriers, wherein the compound of the formula (IV) is greater than 93% by weight stereomerically pure.

10. The method of claim **9**, wherein the compound of formula (IV) is formulated as a tablet.

11. The method of claim **9**, wherein the compound is formulated as a capsule.

12. The method of claim **9**, wherein the compound of formula (IV) is greater than 95% by weight stereomerically pure.

13. The method of claim **9**, wherein the compound of formula (IV) is greater than 97% by weight stereomerically pure.

14. The method of claim **9**, wherein the compound of formula (IV) is substantially free of other diastereomers of the compound.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

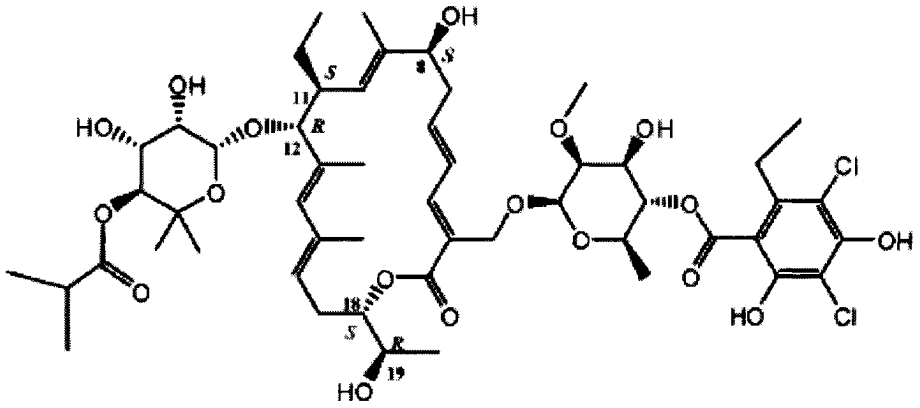
CERTIFICATE OF CORRECTION

PATENT NO. : 7,906,489 B2
APPLICATION NO. : 11/882219
DATED : March 15, 2011
INVENTOR(S) : Youe-Kong Shue et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In claim 1 (column 30, lines 1-36), Formula IV should appear as follows:

(IV)



Signed and Sealed this
Seventh Day of June, 2011

David J. Kappos

David J. Kappos
Director of the United States Patent and Trademark Office

EXHIBIT 2

US008586551B2

(12) **United States Patent**
Shue et al.

(10) **Patent No.: US 8,586,551 B2**
(45) **Date of Patent: *Nov. 19, 2013**

(54) **18-MEMBERED MACROCYCLES AND ANALOGS THEREOF**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 529 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **12/551,056**

(22) Filed: **Aug. 31, 2009**

(65) **Prior Publication Data**

US 2010/0009925 A1 Jan. 14, 2010

Related U.S. Application Data

(63) Continuation of application No. 11/882,219, filed on Jul. 31, 2007, now Pat. No. 7,906,489, which is a continuation-in-part of application No. PCT/US2005/002887, filed on Jan. 31, 2005.

(51) **Int. Cl.**

A01N 43/04	(2006.01)
A61K 31/70	(2006.01)
C07H 1/00	(2006.01)
C07G 3/00	(2006.01)

(52) **U.S. Cl.**
USPC **514/25**; 514/28; 536/1.11; 536/4.1

(58) **Field of Classification Search**
USPC 514/25, 28; 536/1.11, 4.1
See application file for complete search history.

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(57) **ABSTRACT**

The present invention relates generally to the 18-membered macrocyclic antimicrobial agents called Tiacumicins, specifically, OPT-80 (which is composed almost entirely of the R-Tiacumicin B), pharmaceutical compositions comprising OPT-80, and methods using OPT-80. In particular, this compound is a potent drug for the treatment of bacterial infections, specifically *C. difficile* infections.

6 Claims, 1 Drawing Sheet

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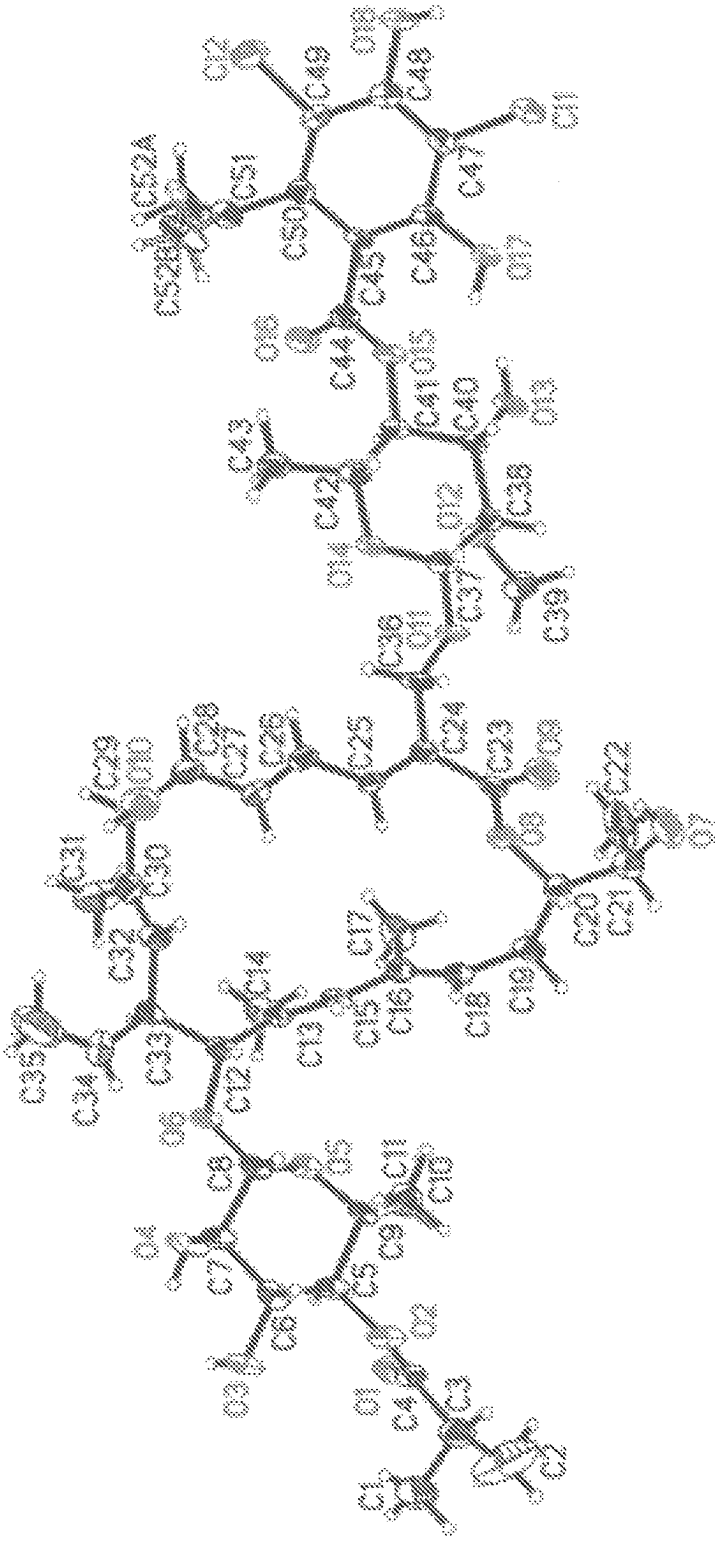


18-MEMBERED MACROCYCLES AND ANALOGS THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. application Ser. No. 11/882,219 (filed Jul. 31, 2007 and now U.S. Pat. No. 7,906,489 granted Mar. 15, 2011) which is a continuation-in-part application of PCT International Application PCT/US2005/002887 (filed Jan. 31, 2005) each of which is incorporated by reference in its entirety.

FIELD OF INVENTION

The present invention relates generally to the 18-membered macrocyclic antimicrobial agents called Tiacumicins, specifically, the R-Tiacumicin B or Tiacumicin B and its related compounds. In particular, substantially pure R-Tiacumicin B, as a potent antibiotic agent for the treatment of bacterial infections, specifically GI infections caused by toxin producing strains of *Clostridium difficile* (*C. difficile*), *Staphylococcus aureus* (*S. aureus*) including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Clostridium perfringens* (*C. perfringens*).

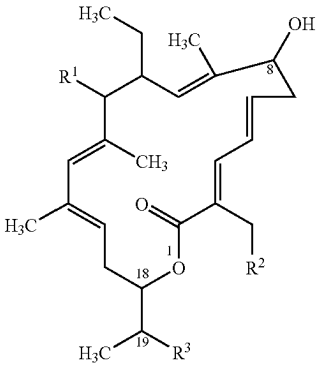
BACKGROUND OF THE INVENTION

Macrocycles are an important therapeutic class of antibiotics. These compounds are frequently produced as a family of closely related biogenetic congeners. The Tiacumicins are

a series of 18-membered macrocyclic antibiotics in which the macrocyclic ring is glycosidically attached to one or two sugars. A seven-carbon sugar is esterfied at various positions with small fatty acids. The other sugar, when present, is esterified with an isomer of the fully substituted benzoic acid, everninic acid. (Journal of Liquid Chromatography, 1988, 11: 191-201).

Tiacumicins are a family of related compounds that contain the 18-membered ring shown in Formula I below.

Formula I



At present, several distinct Tiacumicins have been identified and six of these (Tiacumicin A-F) are defined by their particular pattern of substituents R^1, R^2, and R^3 (U.S. Pat. No. 4,918,174; J. Antibiotics, 1987, 40: 575-588), as shown in Table 1.

TABLE 1

Substituents Present In Tiacumcins A-F

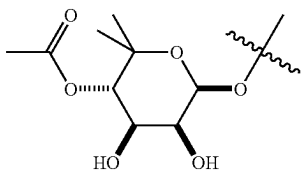
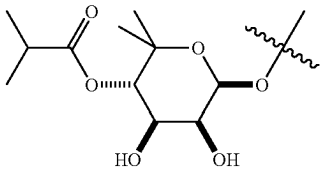
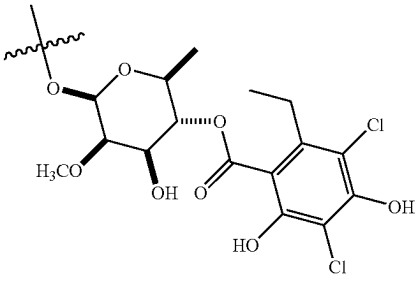
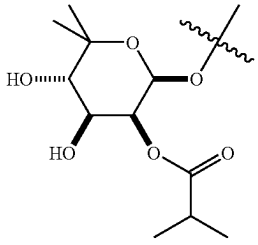
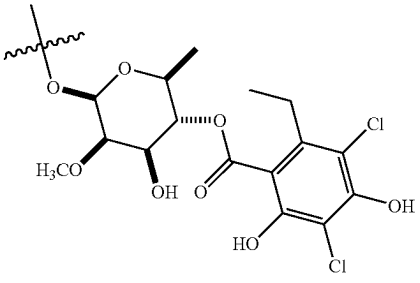
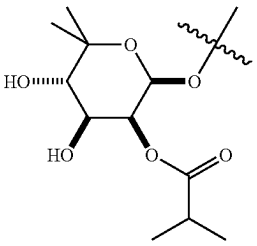
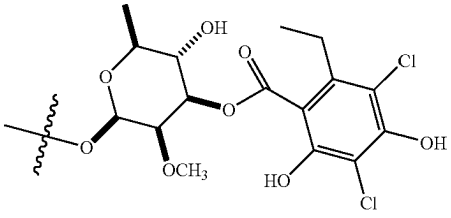
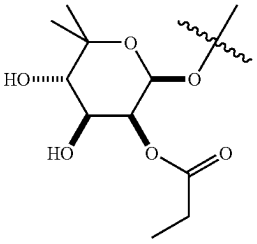
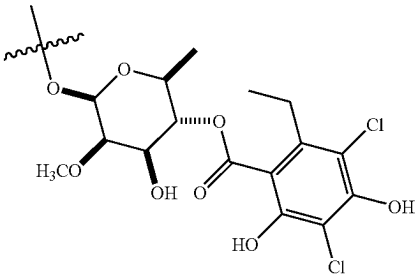
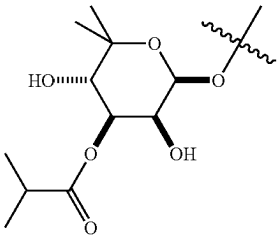
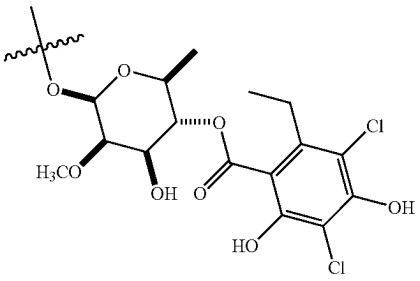
	R^1	R^2	R^3
A		H	H
B			OH
C			OH

TABLE 1-continued

Substituents Present In Tiacumcins A-F

	R^1	R^2	R^3
D			OH
E			OH
F			OH

Tiacumicins A-F have been characterized spectroscopically and by other physical methods. The chemical structures of Tiacumicins are based on spectroscopy: UV-vis, IR and ^1H and ^{13}C NMR, see for example J. Antibiotics, 1987, 40: 575-588. Inspection of Table 1 reveals that certain members of the family are structurally related isomers and/or differ by the presence or absence of certain moieties. Others differ in the nature of their ester groups.

Tiacumicins are produced by bacteria, including *Dactylosporangium aurantiacum* subspecies *hamdenensis*, which may be obtained from the ARS Patent Collection of the Northern Regional Research Center, United States Department of Agriculture, 1815 North University Street, Peoria, Ill. 61604, accession number NRRL 18085. The characteristics of strain AB 718C-41 are given in J. Antibiotics, 1987, 40: 567-574 and U.S. Pat. No. 4,918,174.

C. difficile-associated diarrhea (CDAD) is a disease characterized by severe and painful diarrhea. *C. difficile* is responsible for approximately 20% of the cases of antibiotic-associated diarrhea (AAD) and the majority of the cases of antibiotic-associated colitis (AAC). These diseases are typically caused by toxin producing strains of *C. difficile, S. aureus* including methicillin-resistant *S. aureus* (MRSA) and *Clostridium perfringens* (*C. perfringens*). AAD represents a major economic burden to the healthcare system that is conservatively estimated at $3-6 billion per year in excess hospital costs in the U.S. alone.

Vancomycin-resistant enterococci, for which intestinal colonization provides a constant reservoir for infection, has also emerged as a major nosocomial pathogen associated with increased health care cost and mortality. VRE can appear as coinfection in patients infected with *C. difficile*, or more commonly cause infection in certain high risk patients such as haematology and oncology patients, patients in intensive care units and patients receiving solid organ transplants.

Methicillin-resistant Staphylococci, such as MRSA, are increasing in prevalence in both the hospital and community settings. Staphylococci are found on the skin and within the digestive and respiratory tracts but can infect open wounds and burns and can progress to serious systemic infection. The emergence of multi-drug resistant Staphylococci, especially, in the hospital where antibiotic use is frequent and selective pressure for drug-resistant organisms is high, has proven a challenge for treating these patients. The presence of MRSA on the skin of patients and health care workers promotes transmission of the multi-drug resistant organisms.

Similar diseases, including but not limited to clostridial enterocolitis, neonatal diarrhea, antibiotic-associated enterocolitis, sporadic enterocolitis, and nosocomial enterocolitis are also significant problems in some animal species.

AAD is a significant problem in hospitals and long-term care facilities and in the community. *C. difficile* is the leading cause of AAD in the hospital setting, accounting for approximately 20% of cases of AAD and the majority of cases of

antibiotic-associated colitis (AAC). The rising incidence of *Clostridium difficile*-associated diarrhea (CDAD) has been attributed to the frequent prescription of broad-spectrum antibiotics to hospitalized patients.

The most serious form of the disease is pseudomembranous colitis (PMC), which is manifested histologically by colitis with mucosal plaques, and clinically by severe diarrhea, abdominal cramps, and systemic toxicity. The overall mortality rate from CDAD is low, but is much greater in patients who develop severe colitis or systemic toxicity. A recent study has shown that even when death is not directly attributable to *C. difficile*, the rate of mortality in CDAD patients as compared to case-matched controls is much greater.

Diarrhea and colitis are caused by the elaboration of one or more *C. difficile* toxins. The organism proliferates in the colon in patients who have been given broad-spectrum antibiotics or, less commonly, cancer chemotherapy. CDAD is diagnosed in approximately 20% of hospitalized patients who develop diarrhea after treatment with such agents.

There are currently two dominant therapies for CDAD: vancomycin and metronidazole. Vancomycin is not recommended for first-line treatment of CDAD mainly because it is the only antibiotic active against some serious life-threatening multi-drug resistant bacteria. Therefore, in an effort to minimize the emergence of vancomycin-resistant *Enterococcus* (VRE) or vancomycin-resistant *S. aureus* (VRSA), the medical community discourages the use of this drug except when absolutely necessary.

Metronidazole is recommended as initial therapy out of concern for the promotion and selection of vancomycin resistant gut flora, especially enterococci. Despite reports that the frequency of *C. difficile* resistance may be >6% in some countries, metronidazole remains nearly as effective as vancomycin, is considerably less expensive, and can be used either orally or intravenously. Metronidazole is associated with significant adverse effects including nausea, neuropathy, leukopenia, seizures, and a toxic reaction to alcohol. Furthermore, it is not safe for use in children or pregnant women. Clinical recurrence occurs in up to 20% of cases after treatment with either vancomycin or metronidazole. Therapy with metronidazole has been reported to be an important risk factor for VRE colonization and infection. The current treatment regime against Gastrointestinal infections, e.g., *Clostridium difficile*-1-associated diarrhea (CDAD) is rather cumbersome, requiring up to 500 mg four-times daily for 10 to 14 days. Thus, there is a need for better treatment for cases of CDAD as well as for cases of other Antibiotic-associated diarrhea (AAD) and Antibiotic-associated colitis (AAC).

Tiacumicins, specifically Tiacumicin B, show activity against a variety of bacterial pathogens and in particular against *C. difficile*, a Gram-positive bacterium (Antimicrob. Agents Chemother. 1991, 1108-1111). *C. difficile* is an anaerobic spore-forming bacterium that causes an infection of the bowel. Diarrhea is the most common symptom but abdominal pain and fever may also occur. *C. difficile* is a major causative agent of colitis (inflammation of the colon) and diarrhea that may occur following antibiotic intake. This bacterium is primarily acquired in hospitals and chronic care facilities. Because Tiacumicin B shows promising activity against *C. difficile*, it is expected to be useful in the treatment of bacterial infections, especially those of the gastrointestinal tract, in mammals. Examples of such treatments include but are not limited to treatment of colitis and treatment of irritable bowel syndrome. Tiacumicins may also find use for the treatment of gastrointestinal cancers.

Tiacumicin antibiotics are described in U.S. Pat. No. 4,918,174 (issued Apr. 17, 1990), J. Antibiotics 1987, 40: 575-588, J. Antibiotics 1987, 40: 567-574, J. Liquid Chromatography 1988, 11: 191-201, Antimicrobial Agents and Chemotherapy 1991, 35: 1108-1111,U.S. Pat. No. 5,583,115 (issued Dec. 10, 1996), and U.S. Pat. No. 5,767,096 (issued Jun. 16, 1998), which are all incorporated herein by reference. Related compounds are the Lipiarmycin antibiotics (c.f., J. Chem. Soc. Perkin Trans. I, 1987, 1353-1359 and J. Antibiotics 1988, 41: 308-315) and the Clostomicin antibiotics (J. Antibiotics 1986, 39: 1407-1412), which are all incorporated herein by reference.

SUMMARY OF THE INVENTION

The present invention relates to new pharmaceutical compositions containing R-Tiacumicins, specifically the optically pure R-Tiacumicin B, and to the use of these new compositions in combination with existing drugs to treat infections caused by gram-positive anerobes.

One embodiment of the present invention is directed towards the discovery that the chiral center at C-19 of Tiacumicin B has great effect on biological activity. It has now been discovered that a substantially pure preparation of higher activity R-Tiacumicin B, which has an R-hydroxy group at C-19 has surprisingly lower MIC values than the optically pure S-isomer of Tiacumicin B and other Tiacumicin B related compounds.

In another embodiment of the present invention the substantially pure R-Tiacumicin B has an unusually long post-antibiotic activity (PAE).

This invention encompasses the composition of novel antibiotic agents, containing substantially pure R-Tiacumicins, by submerged aerobic fermentation of the microorganism *Dactylosporangium aurantiacum* subspecies *hamdenensis*. The production method is covered by WO 2004/014295 A2, which is hereby incorporated by reference.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** shows the Oak Ridge Thermal Ellipsoid Plot Program (ORTEP) chemical structure of R-Tiacumicin B.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

The term "antibiotic-associated condition" refers to a condition resulting when antibiotic therapy disturbs the balance of the microbial flora of the gut, allowing pathogenic organisms such as enterotoxin producing strains of *C. difficile, S. aureus* and *C. perfringens* to flourish. These organisms can cause diarrhea, pseudomembranous colitis, and colitis and are manifested by diarrhea, urgency, abdominal cramps, tenesmus, and fever among other symptoms. Diarrhea, when severe, causes dehydration and the medical complications associated with dehydration.

The term "asymmetrically substituted" refers to a molecular structure in which an atom having four tetrahedral valences is attached to four different atoms or groups. The commonest cases involve the carbon atom. In such cases, two optical isomers (D- and L-enantiomers or R- and S-enantiomers) per carbon atom result which are nonsuperposable mirror images of each other. Many compounds have more than one asymmetric carbon. This results in the possibility of

many optical isomers, the number being determined by the formula 2^n, where n is the number of asymmetric carbons.

As used herein, and unless otherwise indicated, the terms "biohydrolyzable carbamate," "biohydrolyzable carbonate," "biohydrolyzable ureide" and "biohydrolyzable phosphate" mean a carbamate, carbonate, ureide and phosphate, respectively, of a compound that either: 1) does not interfere with the biological activity of the compound but can confer upon that compound advantageous properties in vivo, such as uptake, duration of action, or onset of action; or 2) is biologically inactive but is converted in vivo to the biologically active compound. Examples of biohydrolyzable carbamates include, but are not limited to, lower alkylamines, substituted ethylenediamines, aminoacids, hydroxyalkylamines, heterocyclic and heteroaromatic amines, and polyether amines.

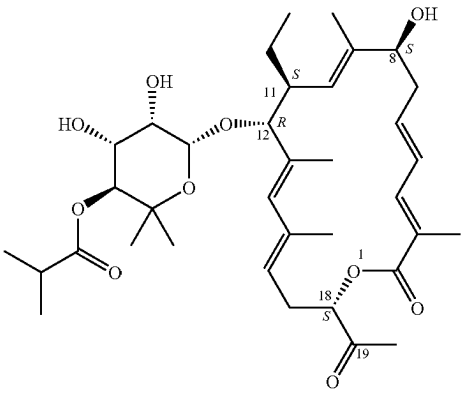
As used herein, and unless otherwise indicated, the term "biohydrolyzable ester" means an ester of a compound that either: 1) does not interfere with the biological activity of the compound but can confer upon that compound advantageous properties in vivo, such as uptake, duration of action, or onset of action; or 2) is biologically inactive but is converted in vivo to the biologically active compound. Examples of biohydrolyzable esters include, but are not limited to, lower alkyl esters, alkoxyacyloxy esters, alkyl acylamino alkyl esters, and choline esters.

As used herein, and unless otherwise indicated, the term "biohydrolyzable amide" means an amide of a compound that either: 1) does not interfere with the biological activity of the compound but can confer upon that compound advantageous properties in vivo, such as uptake, duration of action, or onset of action; or 2) is biologically inactive but is converted in vivo to the biologically active compound. Examples of biohydrolyzable amides include, but are not limited to, lower alkyl amides, .alpha.-amino acid amides, alkoxyacyl amides, and alkylaminoalkylcarbonyl amides.

The term "broth" as used herein refers to the fluid culture medium as obtained during or after fermentation. Broth comprises a mixture of water, the desired antibiotic(s), unused nutrients, living or dead organisms, metabolic products, and the adsorbent with or without adsorbed product.

The term "C-19 Ketone" refers to a Tiacumicin B related compound shown below in Formula II:

Formula II



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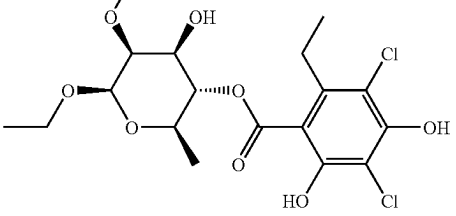


The term "diastereomers" refers to stereoisomers that are not mirror images of each other.

The term "enantiomer" refers to a non-superimposable mirror image of itself. An enantiomer of an optically active isomer rotates plane polarized light in an equal but opposite direction of the original isomer. A solution of equal parts of an optically active isomer and its enantiomer is known as a racemic solution and has a net rotation of plane polarized light of zero. Enantiomers will have the opposite prefixes of each other: D- becomes L- or R- becomes S-. Often only one enantiomer is active in a biological system, because most biological reactions are enzymatic and the enzymes can only attach to one of the enantiomers.

The term "excipient" refers to an inert substance added to a pharmacological composition to further facilitate administration of a compound. Examples of excipients include but are not limited to, calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

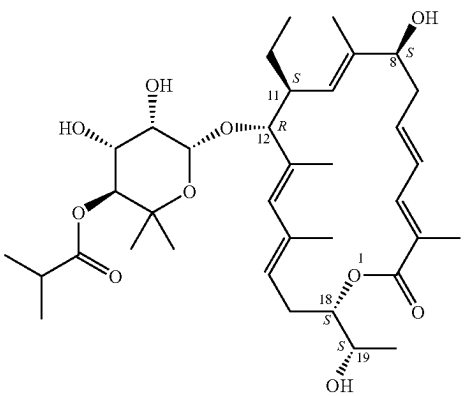
The term "halogen" includes F, Cl, Br and I.

As used herein, the term "hydrate" means a compound of the present invention or a salt thereof that further includes a stoichiometric or non-stoichiometric amount of water bound by non-covalent intermolecular forces.

The term "isomeric mixture" means a mixture of two or more configurationally distinct chemical species having the same chemical formula. An isomeric mixture is a genus comprising individual isomeric species. Examples of isomeric mixtures include stereoisomers (enantiomers and diastereomers), regioisomers, as might result for example from a pericyclic reaction. The compounds of the present invention comprise asymmetrically substituted carbon atoms. Such asymmetrically substituted carbon atoms can result in mixtures of stereoisomers at a particular asymmetrically substituted carbon atom or a single stereoisomer. As a result, racemic mixtures, mixtures of diastereomers, as well as single diastereomers of the compounds of the invention are included in the present invention.

The term "Lipiarmycin A4" refers to a Tiacumicin B related compound shown below in Formula III:

Formula III



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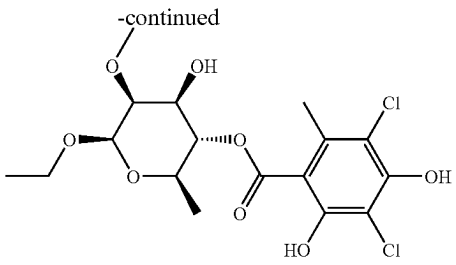


The term "lower alkyl," alone or in combination, refers to an optionally substituted straight-chain or optionally substituted branched-chain having from 1 to about 8 carbons (e.g., C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8), more preferably 1 to 4 carbons (e.g., C_1, C_2, C_3, C_4). Examples of alkyl radicals include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl. A "lower alkyl" is generally a shorter alkyl, e.g. one containing from 1 to about 4 carbon atoms (e.g., C_1, C_2, C_3, C_4).

The term "macrocycles" refers to organic molecules with large ring structures usually containing over 10 atoms.

The term "18-membered macrocycles" refers to organic molecules with ring structures containing 18 atoms.

The term "membered ring" can embrace any cyclic structure, including carbocycles and heterocycles as described above. The term "membered" is meant to denote the number of skeletal atoms that constitute the ring. Thus, for example, pyridine, pyran and thiopyran are 6 membered rings and pyrrole, furan, and thiophene are 5 membered rings.

The term "MIC" or "minimum inhibitory concentration" refers to the lowest concentration of an antibiotic that is needed to inhibit growth of a bacterial isolate in vitro. A common method for determining the MIC of an antibiotic is to prepare several tubes containing serial dilutions of the antibiotic, that are then inoculated with the bacterial isolate of interest. The MIC of an antibiotic can be determined from the tube with the lowest concentration that shows no turbidity (no growth).

The term "MIC_{50}" refers to the lowest concentration of antibiotic required to inhibit the growth of 50% of the bacterial strains tested within a given bacterial species.

The term "MIC_{90}" refers to the lowest concentration of antibiotic required to inhibit the growth of 90% of the bacterial strains tested within a given bacterial species.

The term "OPT-80" refers to a preparation containing R-Tiacumicin B and Tiacumicin B related compounds (including, but not limited to, Tiacumicins, Lipiarmycin A4 and C-19 Ketone). Preparations of this type are described in detail in PCT application PCT/US03/21977, having an international publication number of WO 2004/014295 A2 and which preparations and are incorporated here by reference.

The term "ORTEP" refers to the Oak Ridge Thermal Ellipsoid Plot computer program, written in Fortran, for drawing crystal structure illustrations. Ball-and-stick type illustrations of a quality suitable for publication are produced with either spheres or thermal-motion probability ellipsoids, derived from anisotropic temperature factor parameters, on the atomic sites. The program also produces stereoscopic pairs of illustrations which aid in the visualization of complex arrangements of atoms and their correlated thermal motion patterns.

The term "PAE" or "post-antibiotic effect" refers to a well-established pharmacodynamic parameter that reflects the persistent suppression of bacterial growth following antibiotic exposure.

The term "patient" refers to a human or animal in need of medical treatment. For the purposes of this invention, human patients are typically institutionalized in a primary medical care facility such as a hospital or nursing home. However, treatment of a disease associated with the use of antibiotics or cancer chemotherapies or antiviral therapies can occur on an outpatient basis, upon discharge from a primary care facility, or can be prescribed by a physician for home-care, not in association with a primary medical care facility. Animals in need of medical treatment are typically in the care of a veterinarian.

The term "pharmaceutically acceptable carrier" refers to a carrier or diluent that is pharmaceutically acceptable.

The term "pharmaceutically acceptable salts" refers to those derived from pharmaceutically acceptable inorganic and organic bases. Salts derived from appropriate bases include alkali metal (e.g. sodium or potassium), alkaline earth metal (e.g., magnesium), ammonium and $N(C_1\text{-}C_4\ alkyl)_4^+$ salts, and the like. Illustrative examples of some of these include sodium hydroxide, potassium hydroxide, choline hydroxide, sodium carbonate, and the like. The term "pharmaceutically acceptable salt" also refers to salts prepared from pharmaceutically acceptable non-toxic acids, including inorganic acids and organic acids. Suitable non-toxic acids include inorganic and organic acids such as, but not limited to, acetic, alginic, anthranilic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethenesulfonic, formic, fumaric, furoic, gluconic, glutamic, glucorenic, galacturonic, glycidic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phenylacetic, propionic, phosphoric, salicylic, stearic, succinic, sulfanilic, sulfuric, tartaric acid, p-toluenesulfonic and the like. Particularly preferred are hydrochloric, hydrobromic, phosphoric, and sulfuric acids, and most particularly preferred is the hydrochloride salt.

The term "pharmaceutical composition" refers to a composition of the R-Tiacumicin described herein, or physiologically acceptable salts thereof, with other chemical components, such as physiologically acceptable carriers and/or excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to a mammal, including humans.

The term "physiologically acceptable carrier" refers to a carrier or diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound.

As used herein, and unless otherwise indicated, the term "prodrug" means a derivative of a compound that can hydrolyze, oxidize, or otherwise react under biological conditions (in vitro or in vivo) to provide the compound. Examples of prodrugs include, but are not limited to, compounds that comprise biohydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. Other examples of prodrugs include compounds that comprise —NO, —NO_2, —ONO, or —ONO_2 moieties. When used to describe a compound of the invention, the term "prodrug" may also to be interpreted to exclude other compounds of the invention for example racemates.

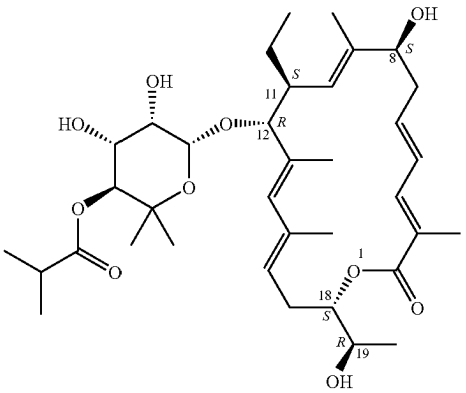
The term "pseudomembranous colitis" or "enteritis" refers to the formation of pseudomembranous material (i.e., material composed of fibrin, mucous, necrotic epithelial cells and leukocytes) due to inflammation of the mucous membrane of both the small and large intestine.

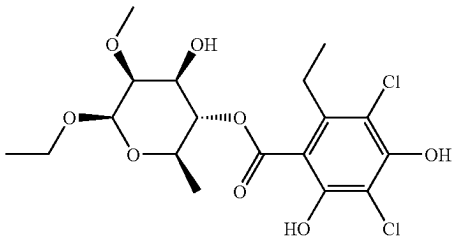
The terms "R" and "S" configuration, as used herein, are as defined by the IUPAC 1974 Recommendations for Section E,

Fundamental Stereochemistry, *Pure Appl. Chem.* (1976) 45, 13-30. Chiral molecules can be named based on the atomic numbers of the atoms or groups of atoms, the ligands that are attached to the chiral center. The ligands are given a priority (the higher the atomic number the higher the priority) and if the priorities increase in a clockwise direction, they are said to be R-. Otherwise, if they are prioritized in a counterclockwise direction they are said to be S-.

The term "R-Tiacumicin B" refers to the optically pure (R)-isomer of Tiacumicin B with an (R)-hydroxy group at C-19, as shown below in Formula IV:

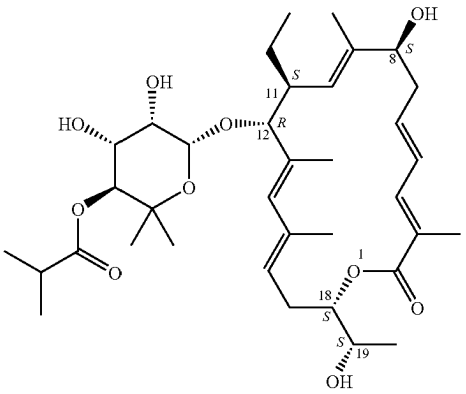
Formula IV





The term "S-Tiacumicin B" refers to the optically pure (S)-isomer of Tiacumicin B with an (S)-hydroxy group at C-19, as shown below in Formula V:

Formula V



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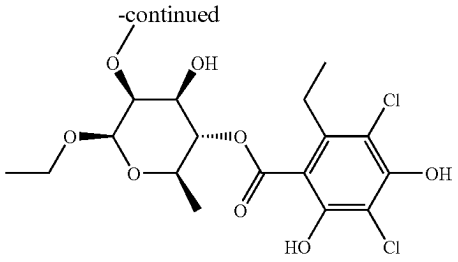


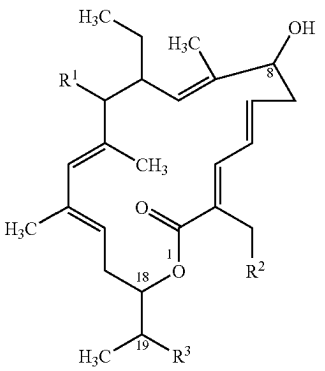
The term "stereoisomers" refers to compounds whose molecules have the same number and kind of atoms and the same atomic arrangement, but differ in their spatial arrangement.

As used herein, and unless otherwise indicated, the terms "optically pure," "stereomerically pure," and "substantially stereomerically pure" are used interchangeably and mean one stereoisomer of a compound or a composition that comprises one stereoisomer of a compound and is substantially free of other stereoisomer(s) of that compound. For example, a stereomerically pure compound or composition of a compound having one chiral center will be substantially free of the opposite enantiomer of the compound. A stereomerically pure compound or composition of a compound having two chiral centers will be substantially free of other diastereomers of the compound. A typical stereomerically pure compound comprises greater than about 80% by weight of one stereoisomer of the compound and less than about 20% by weight of other stereoisomers of the compound, more preferably greater than about 90% by weight of one stereoisomer of the compound and less than about 10% by weight of the other stereoisomers of the compound, even more preferably greater than about 95% by weight of one stereoisomer of the compound and less than about 5% by weight of the other stereoisomers of the compound, and most preferably greater than about 97% by weight of one stereoisomer of the compound and less than about 3% by weight of the other stereoisomers of the compound.

The term "sugar" generally refers to mono-, di- or oligosaccharides. A saccharide may be substituted, for example, glucosamine, galactosamine, acetylglucose, acetylgalactose, N-acetylglucosamine, N-acetyl-galactosamine, galactosyl-N-acetylglucosamine, N-acetylneuraminic acid (sialic acid), etc., as well as sulfated and phosphorylated sugars. For the purposes of this definition, the saccharides are in their pyranose or furanose form.

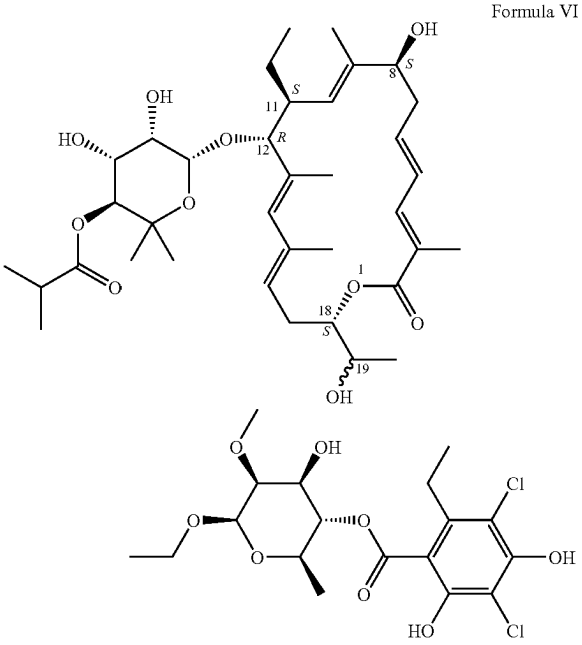
The term "Tiacumicin" as used herein refers to a family of compounds all of which comprise the 18-membered macrocycle shown below in Formula I:

Formula I



The term "Tiacumicin B" as used herein refers to the 18-membered macrocycle shown below in Formula VI:

Formula VI



The term "yield" as used herein refers to an amount of crude Tiacumicin re-constituted in methanol to the same volume as the original fermentation broth. Yield is determined using standard HPLC techniques. Yield is reported in units of mg/L.

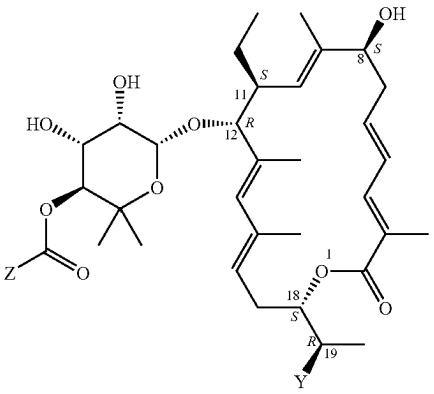
This invention encompasses the composition of novel antibiotic agents, Tiacumicins, by submerged aerobic fermentation of the microorganism *Dactylosporangium aurantiacum* subspecies *hamdenensis*. The production method is covered by WO 2004/014295 A2.

The present invention relates to new antibacterial compositions containing R-Tiacumicins, specifically the R-Tiacumicin B (which has an R-hydroxyl at C-19), and to the use of these new compositions in combination with existing drugs to treat infections caused by gram-positive anerobes.

The present invention further relates to stereoisomerically pure Tiacumicin B, which contains 90-100% of the R-stereoisomer, preferably at least 93% of the R-stereoisomer, more preferably 95% of the R-stereoisomer, even more preferably 99% of the R-stereoisomer.

In accordance with the present invention there are provided compounds with the structure of Formula VII:

Formula VII



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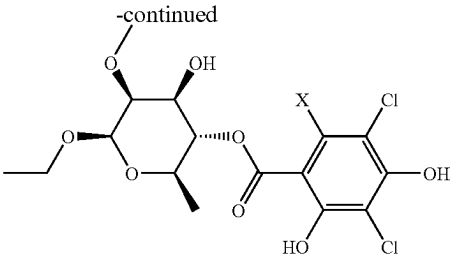


wherein:

X is selected from lower alkyl, and wherein the term "lower alkyl" as used herein refers to branched or straight chain alkyl groups comprising one to two carbon atoms, including methyl, ethyl, n-propyl, isopropyl, and the like; and

Y is selected from OH or a ketone (=O); and

Z is selected from H or lower alkyl, and wherein the term "lower alkyl" as used herein refers to branched or straight chain alkyl groups comprising one to five carbon atoms, including methyl, ethyl, propyl, isopropyl, n-butyl, t-butyl, and the like.

Preferred compounds of the invention are compounds of Formula VII wherein X is methyl or ethyl, Y is ketone (=O) or OH and Z is isopropyl.

More preferred compounds of the invention are the compound of the Formula VII wherein X is ethyl, Y is ketone (=O) or OH and Z is isopropyl.

The most preferred compounds of the invention are the compounds of Formula VII wherein X is ethyl, Y is OH R and Z is isopropyl.

One embodiment of the present invention is directed towards the discovery that the chiral center at C-19 of Tiacumicin B has great effect on biological activity. It has now been discovered that R-Tiacumicin B, which has an R-hydroxy group at C-19 has significantly higher activity than the S-Tiacumicin B and other Tiacumicin B related compounds (Lipiarmycin A4 and C-19 Ketone). The higher activity is shown by much lowered MIC values, which can be seen below in Example 3, Tables 3 and 4 for several strains of *C. difficile, S. aureus, E. faecalis*, and *E. faecium*. This effect of the C-19 chiral center on biological activity is an unexpected and novel discovery.

In another embodiment of the present invention OPT-80 (which is composed almost entirely of the R-Tiacumicin B) has an unusually long post-antibiotic effect (PAE). This is discussed below in Example 4, where it is shown that OPT-80 has a PAE of greater than 24 hours. This PAE is unexpectedly longer than the usual antibiotic PAE of 1-5 hours.

The present invention also relates to the disclosure of pharmaceutical compositions, which comprise a compound of the present invention in combination with a pharmaceutically acceptable carrier.

Yet another aspect of the invention discloses a method of inhibiting or treating bacterial infections in humans, comprising administering to the patient a therapeutically effective amount of a compound of the invention alone or in combination with another antibacterial or antifungal agent.

Production

The 18-membered macrocycles and analogs thereof are produced by fermentation. Cultivation of *Dactylosporangium aurantiacum* subsp. *hamdenensis* AB 718C-41 NRRL 18085 for the production of the Tiacumicins is carried out in a medium containing carbon sources, inorganic salts

and other organic ingredients with one or more absorbents under proper aeration conditions and mixing in a sterile environment.

The microorganism to produce the active antibacterial agents was identified as belonging to the family Actinoplanaceae, genus *Dactylosporangium* (*J. of Antibiotics,* 1987, 40: 567-574 and U.S. Pat. No. 4,918,174). It has been designated *Dactylasporangium aurantiacum* subspecies *hamdenensis* 718C-41. The subculture was obtained from the ARS Patent Collection of the Northern Regional Research Center, United States Department of Agriculture, 1815 North University Street, Peoria, Ill. 61604, U.S.A., where it was assigned accession number NRRL 18085. The characteristics of strain AB 718C-41 are given in the Journal of Antibiotics, 1987, 40: 567-574 and U.S. Pat. No. 4,918,174.

Methods of isolating stereomerically pure isomers are known in the art. Methods of isolating stereomerically pure R-Tiacumicin include, but are not limited to, recrystallization of the crude mixture in solvents including, aqueous methanol or isopropanol and chiral HPLC.

This invention encompasses the composition of novel antibiotic agents, Tiacumicins, by submerged aerobic fermentation of the microorganism *Dactylosporangium aurantiacum* subspecies *hamdenensis*. The production method is covered by WO 2004/014295 A2, which is hereby incorporated by reference.

Pharmaceutical Formulation and Administration

Pharmaceutical compositions of the Tiacumicin compounds of the present invention, specifically OPT-80 (which is composed almost entirely of the R-Tiacumicin), according to the invention may be formulated to release an antibiotic substantially immediately upon administration or at any predetermined time or time period after administration.

The latter types of compositions are generally known as modified release formulations, which include formulations that create a substantially constant concentration of the drug within the intestinal tract over an extended period of time, and formulations that have modified release characteristics based on temporal or environmental criteria as described in Modified-Release Drug Delivery Technology, ed. M. J. Rathbone, J. Hodgraft and M. S. Roberts. Marcel Dekker, Inc. New York.

Any oral biologically-acceptable dosage form, or combinations thereof, can be employed in the methods of the invention. Examples of such dosage forms include, without limitation, chewable tablets, quick dissolve tablets, effervescent tablets, reconstitutable powders, elixirs, liquids, suppositories, creams, solutions, suspensions, emulsions, tablets, multi-layer tablets, bi-layer tablets, capsules, soft gelatin capsules, hard gelatin capsules, osmotic tablets, osmotic capsules, caplets, lozenges, chewable lozenges, beads, powders, granules, particles, microparticles, dispersible granules, ingestibles, infusions, health bars, confections, animal feeds, cereals, cereal coatings, foods, nutritive foods, functional foods and combinations thereof. The preparation of any of the above dosage forms is well known to persons of ordinary skill in the art. Additionally, the pharmaceutical formulations may be designed to provide either immediate or controlled release of the antibiotic upon reaching the target site. The selection of immediate or controlled release compositions depends upon a variety of factors including the species and antibiotic susceptibility of Gram-positive bacteria being treated and the bacteriostatic/bactericidal characteristics of the therapeutics. Methods well known in the art for making formulations are found, for example, in Remington: The Science and Practice of Pharmacy (20th ed.), ed. A. R. Gennaro, 2000, Lippincott Williams & Wilkins, Philadelphia, or in Encyclopedia of Pharmaceutical Technology, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York.

Immediate release formulations for oral use include tablets or capsules containing the active ingredient(s) in a mixture with non-toxic pharmaceutically acceptable excipients. These excipients may be, for example, inert diluents or fillers (e.g., sucrose, sorbitol, sugar, mannitol, microcrystalline cellulose, starches including potato starch, calcium carbonate, sodium chloride, lactose, calcium phosphate, calcium sulfate, or sodium phosphate); granulating and disintegrating agents (e.g., cellulose derivatives including microcrystalline cellulose, starches including potato starch, croscarmellose sodium, alginates, or alginic acid); binding agents (e.g., sucrose, glucose, mannitol, sorbitol, acacia, alginic acid, sodium alginate, gelatin, starch, pregelatinized starch, microcrystalline cellulose, magnesium aluminum silicate, carboxymethylcellulose sodium, methylcellulose, hydroxypropyl methylcellulose, ethylcellulose, polyvinylpyrrolidone, or polyethylene glycol); and lubricating agents, glidants, and antiadhesives (e.g., magnesium stearate, zinc stearate, stearic acid, silicas, hydrogenated vegetable oils, or talc). Other pharmaceutically acceptable excipients can be colorants, flavoring agents, plasticizers, humectants, buffering agents, and the like as are found, for example, in The Handbook of Pharmaceutical Excipients, third edition, edited by Arthur H. Kibbe, American Pharmaceutical Association Washington D.C.

Dissolution or diffusion controlled release can be achieved by appropriate coating of a tablet, capsule, pellet, or granulate formulation of compounds, or by incorporating the compound into an appropriate matrix. A controlled release coating may include one or more of the coating substances mentioned above and/or, e.g., shellac, beeswax, glycowax, castor wax, carnauba wax, stearyl alcohol, glyceryl monostearate, glyceryl distearate, glycerol palmitostearate, ethylcellulose, acrylic resins, dl-polylactic acid, cellulose acetate butyrate, polyvinyl chloride, polyvinyl acetate, vinyl pyrrolidone, polyethylene, polymethacrylate, methylmethacrylate, 2-hydroxymethacrylate, methacrylate hydrogels, 1,3 butylene glycol, ethylene glycol methacrylate, and/or polyethylene glycols. In a controlled release matrix formulation, the matrix material may also include, e.g., hydrated methylcellulose, carnauba wax and stearyl alcohol, carbopol 934, silicone, glyceryl tristearate, methyl acrylate-methyl methacrylate, polyvinyl chloride, polyethylene, and/or halogenated fluorocarbon.

A controlled release composition may also be in the form of a buoyant tablet or capsule (i.e., a tablet or capsule that, upon oral administration, floats on top of the gastric content for a certain period of time). A buoyant tablet formulation of the compound(s) can be prepared by granulating a mixture of the antibiotic with excipients and 20-75% w/w of hydrocolloids, such as hydroxyethylcellulose, hydroxypropylcellulose, or hydroxypropyl-methylcellulose. The obtained granules can then be compressed into tablets. On contact with the gastric juice, the tablet forms a substantially water-impermeable gel barrier around its surface. This gel barrier takes part in maintaining a density of less than one, thereby allowing the tablet to remain buoyant in the gastric juice. Other useful controlled release compositions are known in the art (see, for example, U.S. Pat. Nos. 4,946,685 and 6,261,601).

A modified release composition may be comprised of a compression-coated core whose geometric configuration controls the release profile of the encapsulated antibiotic. By varying the geometry of the core, the profile of the antibiotic release can be adjusted to follow zero order, first order or a combination of these orders. The system can also be designed

to deliver more beneficial agents at the same time, each having a different release profile (see, for example U.S. Pat. Nos. 4,111,202 and 3,279,995).

Formulations that target the Tiacumicin compounds of the present invention, specifically OPT-80 (which is composed almost entirely of the R-Tiacumicin), that release to particular regions of the intestinal tract can also be prepared. The Tiacumicin compounds of the present invention, specifically OPT-80, can be encapsulated in an enteric coating that prevents release degradation and release from occurring in the stomach, but dissolves readily in the mildly acidic or neutral pH environment of the small intestine. A formulation targeted for release of antibiotic to the colon, utilizing technologies such as time-dependent, pH-dependent, or enzymatic erosion of polymer matrix or coating can also be used.

The targeted delivery properties of the Tiacumicin compounds of the present invention, specifically OPT-80 (which is composed almost entirely of the R-Tiacumicin B), containing formulation may be modified by other means. For example, the antibiotic may be complexed by inclusion, ionic association, hydrogen bonding, hydrophobic bonding, or covalent bonding. In addition polymers or complexes susceptible to enzymatic or microbial lysis may also be used as a means to deliver drug.

Microsphere encapsulation of the Tiacumicin compounds of the present invention, specifically OPT-80 (which is composed almost entirely of the R-Tiacumicin B), is another useful pharmaceutical formulation for targeted antibiotic release. The antibiotic-containing microspheres can be used alone for antibiotic delivery, or as one component of a two-stage release formulation. Suitable staged release formulations may consist of acid stable microspheres, encapsulating the compounds of the present invention, specifically OPT-80 (which is composed almost entirely of the R-Tiacumicin B), to be released later in the lower intestinal tract admixed with an immediate release formulation to deliver antibiotic to the stomach and upper duodenum.

Microspheres can be made by any appropriate method, or from any pharmaceutically acceptable material. Particularly useful are proteinoid microspheres (see, for example, U.S. Pat. No. 5,601,846, or 5,792,451) and PLGA-containing microspheres (see, for example, U.S. Pat. No. 6,235,224 or 5,672,659). Other polymers commonly used in the formation of microspheres include, for example, poly-ϵ-caprolactone, poly(e~caprolactone-Co-DL-lactic acid), poly(DL-lactic acid), poly(DL-lactic acid-Co-glycolic acid) and poly(s-caprolactone-Co-glycolic acid) (see, for example, Pitt et al., J. Pharm. Sci., 68:1534, 1979). Microspheres can be made by procedures well known in the art including spray drying, coacervation, and emulsification (see for example Davis et al. Microsphere and Drug Therapy, 1984, Elsevier; Benoit et al. Biodegradable Microspheres: Advances in Production Technologies, Chapter 3, ed. Benita, S, 1996, Dekker, New York; Microencapsulation and Related Drug Processes, Ed. Deasy, 1984, Dekker, New York; U.S. Pat. No. 6,365,187).

Powders, dispersible powders, or granules suitable for preparation of aqueous solutions or suspensions of the Tiacumicin compounds of the present invention, specifically OPT-80 (which is composed almost entirely of the R-Tiacumicin B), by addition of water are convenient dosage forms for oral administration. Formulation as a suspension provides the active ingredient in a mixture with a dispersing or wetting agent, suspending agent, and one or more preservatives. Suitable dispersing or wetting agents are, for example, naturally-occurring phosphatides (e.g., lecithin or condensation products of ethylene oxide with a fatty acid, a long chain aliphatic alcohol, or a partial ester derived from fatty acids) and a hexitol or a hexitol anhydride (e.g., polyoxyethylene stearate, polyoxyethylene sorbitol monooleate, polyoxyethylene sorbitan monooleate, and the like). Suitable suspending agents are, for example, sodium carboxymethylcellulose, methylcellulose, sodium alginate, and the like.

EXAMPLES

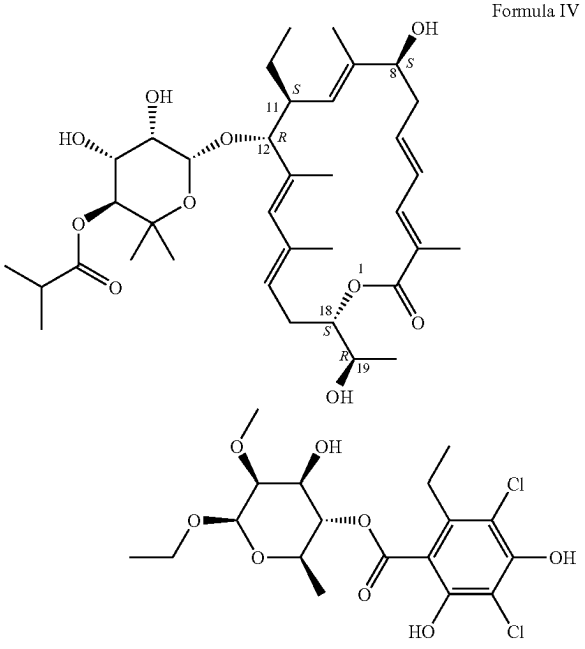
The following examples are provided by way of describing specific embodiments of the present invention without intending to limit the scope of the invention in any way.

Example 1

Exact Structure of R-Tiacumicin B

The exact structure of the R-Tiacumicin B (the major most active component of OPT-80) is shown below in Formula IV. The X-ray crystal structure of the R-Tiacumicin B was obtained from a colorless, parallelepiped-shaped crystal (0.08×0.14×0.22 mm) grown in methanol and is shown as an ORTEP diagram in FIG. **1**. This x-ray structure confirms the structure shown below in Formula IV. The official chemical name is 3-[[[6-Deoxy-4-O-(3,5-dichloro-2-ethyl-4,6-dihydroxybenzoyl)-2-O-methyl-β-D-mannopyranosyl]oxy]-methyl]-12(R)-[[6-deoxy-5-C-methyl-4-O-(2-methyl-1-oxopropyl)-β-D-lyxo-hexopyranosyl]oxy]-11(S)-ethyl-8(S)-hydroxy-18(S)-(1(R)-hydroxyethyl)-9,13,15-trimethyloxacyclooctadeca-3,5,9,13,15-pentaene-2-one.

Formula IV



Example 2

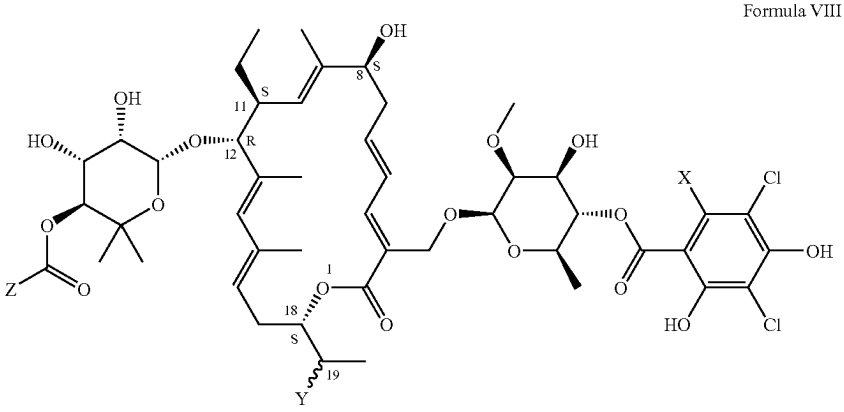
Analytical Data of OPT-80 and Related Substances

The analytical data of OPT-80 (which is composed almost entirely of the R-Tiacumicin B, which is the most active component of OPT-80) and three related compounds (S-Tiacumicin B, Lipiarmycin A4, and C-19 ketone) are summarized below. The structures of these compounds are shown in Formula VIII and Table 2 below.

TABLE 2

Structure of R-Tiacumicin B (the major most active component of OPT-80) and related substances

Formula VIII



Compound	X	Y	Z
R-Tiacumicin B	Ethyl	(R)-OH	Isopropyl
S-Tiacumicin B	Ethyl	(S)-OH	Isopropyl
Lipiarmycin A4	Methyl	(S)-OH	Isopropyl
C-19 Ketone	Ethyl	=O	Isopropyl

Analytical Data of R-Tiacumicin B

mp 166-169° C. (white needle from isopropanol);

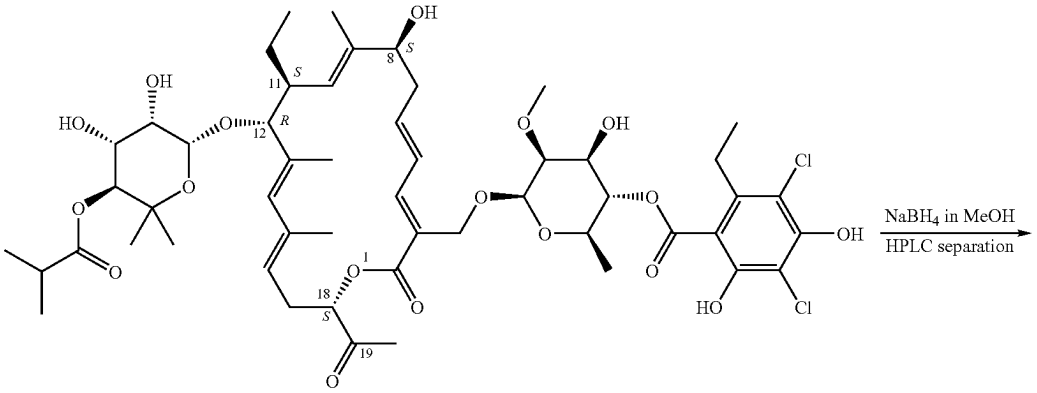
$[\alpha]D^{20}$-6.9 (c 2.0, MeOH);

MS m/z (ESI) 1079.7 $(M+Na)^+$;

^1H ^1H NMR NMR (400 MHz, CD_3OD) δ 7.21 (d, 1H), 6.59 (dd, 1H), 5.95 (ddd, 1H), 5.83 (br s, 1H), 5.57 (t, 1H), 5.13 (br d, 1H), 5.09 (t, 1H), 5.02 (d, 1H), 4.71 (m, 1H), 4.71 (br s, 1H), 4.64 (br s, 1H), 4.61 (d, 1H), 4.42 (d, 1H), 4.23 (m, 1H), 4.02 (pentet, 1H), 3.92 (dd, 1H), 3.73 (m, 2H), 3.70 (d, 1H), 3.56 (s, 3H), 3.52-3.56 (m, 2H), 2.92 (m, 2H), 2.64-2.76 (m, 3H), 2.59 (heptet, 1H), 2.49 (ddd, 1H), 2.42 (ddd, 1H), 2.01 (dq, 1H), 1.81 (s, 3H), 1.76 (s, 3H), 1.65 (s, 3H), 1.35 (d, 3H), 1.29 (m, 1H), 1.20 (t, 3H), 1.19 (d, 3H), 1.17 (d, 3H), 1.16 (d, 3H), 1.14 (s, 3H), 1.12 (s, 3H), 0.87 (t, 3H);

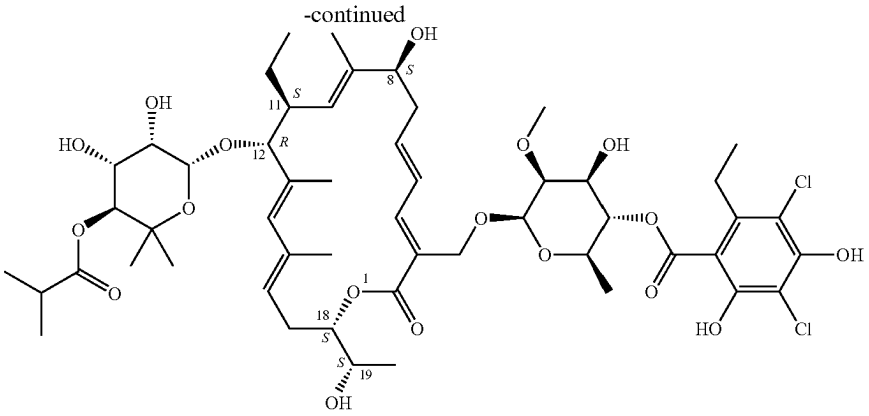
^{13}C NMR (100 MHz, CD_3OD) δ 178.4, 169.7, 169.1, 154.6, 153.9, 146.2, 143.7, 141.9, 137.1, 137.0, 136.4, 134.6, 128.5, 126.9, 125.6, 124.6, 114.8, 112.8, 108.8, 102.3, 97.2, 94.3, 82.5, 78.6, 76.9, 75.9, 74.5, 73.5, 73.2, 72.8, 71.6, 70.5, 68.3, 63.9, 62.2, 42.5, 37.3, 35.4, 28.7, 28.3, 26.9, 26.4, 20.3, 19.6, 19.2, 18.7, 18.2, 17.6, 15.5, 14.6, 14.0, 11.4.

Analytical Data of the S-Tiacumicin B



Formula II (C-19 Ketone)

-continued



Formula V (S-Tiacumicin B)

$NaBH_4$ (9 eq, 48 mg) was added in three portions to a solution of C-19 Ketone (150 mg) in 3 mL MeOH. After 1 h, saturated NH_4Cl solution was added. The mixture was extracted with $CHCl_3$, and then concentrated. S-Tiacumicin B was purified by YMC-pack ODS-A 75×30 mm I.D. column (H_2O:MeOH:AcOH 28:72:1) yielding pure 35 mg of pure S-Tiacumicin B.

MS m/z 1074.5 $(M+NH_4)^+$;

^1H NMR (400 MHz, $CDCl_3$) δ 7.15 (d, J=11.4 Hz, 1H), 6.58 (dd, J=14.1, 11.4 Hz, 1H), 5.82 (ddd, J=14.1, 10.6, 3.5 Hz, 1H), 5.78 (s, 1H), 5.40 (dd, J=7.8, 7.8 Hz, 1H), 5.15 (dd, J=9.5, 9.5 Hz, 1H), 5.01 (d, J=9.9 Hz, 1H), 5.01 (d, J=9.9 Hz, 1H), 4.77 (ddd, J=5.8, 5.3, 5.3 Hz, 1H), 4.68 (d, J=11.6 Hz, 1H), 4.65 (br s, 1H), 4.62 (br s, 1H), 4.42 (d, J=11.6 Hz, 1H), 4.28 (br s, 1H), 4.07-3.97 (m, 2H), 3.74-3.58 (m, 4H), 3.61 (s, 3H), 3.52 (dq, J=9.5, 5.8 Hz, 1H), 3.08 (dq, J=12.6, 6.1 Hz, 1H), 3.01 (dq, J=12.6, 6.1 Hz, 1H), 2.77-2.65 (m, 2H), 2.60 (heptet, J=6.9 Hz, 1H), 2.55-2.44 (m, 3H), 1.95-1.84 (m, 1H), 1.80 (s, 3H), 1.76 (s, 3H), 1.66 (s, 3H), 1.34 (d, J=5.8 Hz, 3H), 1.29-1.24 (m, 1H), 1.27 (d, J=6.6 Hz, 3H), 1.21 (t, J=6.1 Hz, 3H), 1.19 (d, J=6.9 Hz, 3H), 1.18 (d, J=6.9 Hz, 3H), 1.15 (s, 3H), 1.10 (s, 3H), 0.84 (t, J=7.2 Hz, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 177.4, 170.1, 168.8, 157.6, 152.8, 144.4, 143.1, 141.1, 136.7, 136.2, 134.9, 133.8, 128.7, 125.7, 125.2, 123.0, 113.9, 107.5, 107.2, 101.7, 94.9, 92.6, 80.8, 79.2, 76.6, 74.8, 73.5, 72.7, 71.9, 71.7, 70.2, 70.1, 69.5, 63.5, 62.3, 41.5, 36.6, 34.3, 29.5, 28.2, 26.2, 26.0, 19.4, 19.3, 18.9, 18.5, 17.8, 17.3, 15.3, 14.1, 13.7, 11.1;

Analytical Data of Lipiarmycin A_4

MS m/z 1060.5 $(M+NH_4)^+$;

^1H NMR (400 MHz, $CDCl_3$) δ 7.12 (d, J=11.6 Hz, 1H), 6.59 (dd, J=14.1, 11.6 Hz, 1H), 5.85 (br s, 1H), 5.83 (ddd, J=14.1, 10.6, 4.8 Hz, 1H), 5.47 (dd, J=8.3, 8.3 Hz, 1H), 5.12 (dd, J=9.6, 9.6 Hz, 1H), 5.00 (d, J=10.1 Hz, 1H), 4.98 (br d, J=10.6 Hz, 1H), 4.75-4.69 (m, 1H), 4.68 (d, J=11.4 Hz, 1H), 4.66 (br s, 1H), 4.62 (br s, 1H), 4.40 (d, J=11.4 Hz, 1H), 4.26 (br s, 1H), 4.07-4.00 (m, 1H), 4.02 (br d, J=3.3 Hz, 1H), 3.75-3.61 (m, 4H), 3.62 (s, 3H), 3.55 (dq, J=9.6, 6.1 Hz, 1H), 2.82-2.45 (m, 6H), 2.60 (s, 3H), 2.07-1.97 (m, 1H), 1.92 (s, 3H), 1.81 (s, 3H), 1.67 (s, 3H), 1.32 (d, J=6.1 Hz, 3H), 1.30-1.22 (m, 1H), 1.21 (d, J=6.6 Hz, 3H), 1.19 (d, J=7.1 Hz, 3H), 1.18 (d, J=7.1 Hz, 3H), 1.15 (s, 3H), 1.10 (s, 3H), 0.83 (t, J=7.2 Hz, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 177.4, 170.5, 168.9, 157.8, 153.0, 144.3, 140.9, 137.7, 137.0, 136.3, 134.6, 134.4, 129.1, 127.9, 125.3, 123.2, 114.5, 107.4, 107.0, 101.8, 94.7, 92.5, 80.3, 79.6, 76.7, 74.9, 73.5, 72.7, 71.9, 71.6, 70.2, 70.1, 69.1, 63.6, 62.3, 41.9, 36.9, 34.4, 28.8, 28.2, 25.9, 20.0, 19.3, 19.0, 18.6, 18.5, 17.8, 17.2, 15.5, 13.8, 11.2;

Analytical Data of C-19 Ketone

MS m/z 1072.5 $(M+NH_4)^+$;

^1H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, J=11.4 Hz, 1H), 6.61 (dd, J=14.7, 11.4 Hz, 1H), 5.91 (ddd, J=14.7, 9.1, 5.8 Hz, 1H), 5.83 (s, 1H), 5.31 (dd, J=7.9, 7.9 Hz, 1H), 5.14 (dd, J=9.7, 9.7 Hz, 1H), 5.06 (d, J=10.6 Hz, 1H), 5.00 (d, J=10.1 Hz, 1H), 4.98 (dd, J=7.1, 4.8 Hz, 1H), 4.67 (d, J=11.9 Hz, 1H), 4.66 (br s, 1H), 4.61 (br s, 1H), 4.42 (d, J=11.9 Hz, 1H), 4.30 (br s, 1H), 4.02 (br d, J=3.3 Hz, 1H), 3.63-3.60 (m, 4H), 3.62 (s, 3H), 3.51 (dq, J=9.7, 6.1 Hz, 1H), 3.09 (dq, J=14.4, 7.3 Hz, 1H), 3.03 (dq, J=14.4, 7.3 Hz, 1H), 2.76-2.50 (m, 6H), 2.21 (s, 3H), 1.93-1.87 (m, 1H), 1.87 (s, 3H), 1.75 (s, 3H), 1.63 (s, 3H), 1.32 (d, J=6.1 Hz, 3H), 1.27-1.22 (m, 1H), 1.21 (t, J=7.3 Hz, 3H), 1.19 (d, J=7.1 Hz, 3H), 1.18 (d, J=7.1 Hz, 3H), 1.14 (s, 3H), 1.10 (s, 3H), 0.84 (t, J=7.3 Hz, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 205.5, 177.4, 170.1, 166.9, 157.6, 152.8, 145.7, 143.1, 142.0, 137.1, 136.8, 135.5, 133.7, 128.3, 124.8, 124.0, 122.8, 113.9, 107.3, 107.2, 101.3, 94.8, 92.4, 80.4, 77.7, 76.6, 74.7, 73.5, 72.6, 71.8, 71.7, 70.2, 70.0, 63.0, 62.3, 41.5, 36.5, 34.3, 29.6, 28.1, 26.2, 26.1, 26.0, 19.2, 18.9, 18.5, 17.8, 17.3, 15.2, 14.0, 13.3, 11.0

Example 3

Biological Activity

MIC Values Determined for Several *C. Difficile* Strains

OPT-80 (which is composed almost entirely of the R-Tiacumicin B) and its related compounds were tested against *C. difficile*. The MIC values are reported below in Table 3. OPT-80 was surprisingly active when compared to its enantiomer S-Tiacumicin B and Lipiarmycin A4.

TABLE 3

MIC (μg/ml) versus *C. difficile* strains

C. difficile strains	R-Tiacumicin B (>90% Stereomerically Pure)	S-Tiacumicin B	Lipiarmycin A4	C-19 Ketone
ATCC 9689	0.03	0.125	0.06	0.06
ATCC 43255	0.125	1	0.5	0.5
ATCC 17857	0.03	0.25	0.06	nd
LC # 1 (Clinical isolate)	0.125	1	0.5	0.5

MIC Values Determined for Various Microorganisms

OPT-80 (which is composed almost entirely of the R-Tiacumicin B) and its related compounds were tested against several other pathogens. The MIC values are reported below in Table 4. OPT-80 was surprisingly active when compared to S-Tiacumicin B and Lipiarmycin A4.

TABLE 4

MIC (μg/ml) against other microorganisms

Strain ID #	Organism	R-Tiacumicin B (>90% Stereomerically Pure)	S-Tiacumicin B	Lipiarmycin A4
1	*S. aureus* (ATCC 29213)	4	64	8
2	*S. aureus*, (MRSA)	4	64	16
3	*S. aureus*, (MRSA)	4	64	8
4	*E. faecalis* (ATCC 29212)	2	8	2
5	*E. faecalis* Vanc. resistant	4	32	16
6	*E. faecalis* Vanc. resistant	1	16	4

TABLE 4-continued

MIC (μg/ml) against other microorganisms

Strain ID #	Organism	R-Tiacumicin B (>90% Stereomerically Pure)	S-Tiacumicin B	Lipiarmycin A4
7	*E. faecium* Vanc. resistant	1	8	4
8	*E. faecium* Vanc. resistant	1	32	32

Example 4

Post-Antibiotic Effect of OPT-80 in *C. difficile*

The post-antibiotic effect (PAE) of OPT-80 (which is composed almost entirely of the R-Tiacumicin B) was measured versus two strains of *C. difficile*, ATCC 43255 and a clinical isolate, LC3. Vancomycin and rifampin were tested additionally versus LC3.

The PAE at 4× the MIC was observed to be extremely long: greater than 24 hours, for both strains. Because of the long duration of this effect, an exact PAE was not calculated. Vancomycin, on the other hand, had a more normal PAE of less than an hour when used at 4× the MIC versus strain LC3.

Example 5

In Vitro Activity of OPT-80

The in vitro efficacy of OPT-80 (which is composed almost entirely of the R-Tiacumicin B), metronidazole, and vancomycin were assessed versus 110 genetically distinct clinical isolates of *C. difficile* via agar dilution. The MIC data are presented in Tables 5 and 6.

TABLE 5

Geometric mean, MIC ranges, MIC_{50}, and MIC_{90} values for OPT-80 against 110 *C. difficile* clinical isolates, vancomycin, and metronidazole, in μg/mL.

	Range	Geometric Mean	MIC_{50}	MIC_{90}
OPT-80	0.015-0.25	0.08	0.125	0.125
Metronidazole	0.025-0.5	0.15	0.125	0.25
Vancomycin	0.06-4	0.8	1	1

TABLE 6

Raw MIC data for OPT-80, vancomycin (VAN), and metronidazole (MTZ) versus 110 clinical isolates of *C. difficile*, in μg/mL.

ORG ID	R-Tiacumicin B (>90% Stereomerically Pure)	MTZ	VAN	ORG ID	R-Tiacumicin B (>90% Stereomerically Pure)	MTZ	VAN
A1 1535	0.125	0.25	1	CO1 4652	0.25	0.125	1
B1 832	0.06	0.125	1	CP1 5491	0.125	0.25	1
D1 1360	0.03	0.25	1	61 5930	0.03	0.25	1
E1 816	0.06	0.125	1	63 6029	0.25	0.25	0.06
F1 1015	0.125	0.125	1	64 5940	0.125	0.25	1

TABLE 6-continued

Raw MIC data for OPT-80, vancomycin (VAN), and metronidazole (MTZ) versus 110 clinical isolates of *C. difficile*, in μg/mL.

ORG ID	R-Tiacumicin B (>90% Stereomerically Pure)	MTZ	VAN	ORG ID	R-Tiacumicin B (>90% Stereomerically Pure)	MTZ	VAN
G1 1077	0.125	0.125	1	65 5967	0.06	0.25	0.5
I1 1389	0.125	0.125	1	66 6366	0.015	0.125	0.5
J1 5971	0.06	0.25	1	67 6367	0.125	0.25	1
J7 4224	0.03	0.125	1	68 6368	0.03	0.125	0.06
J9 4478	0.06	0.125	1	69 6370	0.25	0.25	0.5
K1 4305	0.125	0.25	0.5	70 6376	0.125	0.25	2
K14 5780	0.125	0.125	1	71 6379	0.125	0.25	1
L1 1423	0.125	0.125	0.5	72 6380	0.125	0.25	2
N1 471	0.125	0.125	0.5	73 6382	0.25	0.25	1
O1 1861	0.06	0.125	1	75 6388	0.125	0.125	0.5
R1 397	0.125	0.125	1	76 6389	0.125	0.25	0.5
R6 6015	0.015	0.25	2	77 6390	0.06	0.125	1
V1 1521	0.125	0.125	0.5	78 6392	0.015	0.03	0.5
W1 3931	0.125	0.5	1	80 6327	0.125	0.125	0.5
X1 1890	0.125	0.125	1	81 6328	0.125	0.125	0.5
Y1 5639	0.06	0.125	0.5	82 6329	0.06	0.03	0.5
Y2 1459	0.06	0.125	1	83 6330	0.06	0.125	0.5
Z1 3036	0.03	0.125	1	84 6331	0.125	0.25	0.5
AA2 4380	0.015	0.125	1	85 6332	0.06	0.125	1
AB2 1725	0.06	0.125	1	86 6333	0.03	0.125	0.5
AC1 1546	0.06	0.125	1	87 6334	0.125	0.125	0.5
AF1 1808	0.125	0.125	0.5	88 6335	0.125	0.25	0.5
AG1 3044	0.125	0.125	1	89 6336	0.25	0.5	1
AH1 3430	0.125	0.25	0.5	90 6338	0.125	0.125	1
AJ1 1557	0.06	0.125	1	91 6339	0.125	0.125	1
AL1 1753	0.06	0.125	0.5	93 6341	0.125	0.125	1
AN1 464	0.125	0.125	0.5	94 6343	0.015	0.06	0.5
AO1 287	0.125	0.125	1	95 6347	0.125	0.125	1
AS1 4099	0.125	0.125	1	96 6348	0.06	0.125	0.5
AT1 1216	0.125	0.125	1	97 6349	0.25	0.125	1
AV1 941	0.25	0.125	0.5	98 6350	0.125	0.5	1
CJ1 893	0.125	0.025	1	101 6354	0.015	0.06	1
AW1 4501	0.125	0.125	1	102 6355	0.016	0.125	1
BE1 4307	0.125	0.25	1	103 6068	0.06	0.125	1
BH1 4506	0.06	0.06	0.5	104 6060	0.03	0.25	1
BI1 1675	0.125	0.125	1	105 6071	0.03	0.125	0.5

TABLE 6-continued

Raw MIC data for OPT-80, vancomycin (VAN), and metronidazole (MTZ) versus 110 clinical isolates of *C. difficile*, in μg/mL.

ORG ID	R-Tiacumicin B (>90% Stereomerically Pure)	MTZ	VAN	ORG ID	R-Tiacumicin B (>90% Stereomerically Pure)	MTZ	VAN
BK1 4291	0.125	0.125	0.5	106 6078	0.03	0.25	0.5
BL1 716	0.125	0.125	1	107 6079	0.06	0.125	0.5
BM1 1453	0.06	0.125	1	109 6274	0.015	0.125	1
BN1 1322	0.125	0.25	1	111 6279	0.03	0.125	1
BR1 1321	0.06	0.125	1	112 6280	0.06	0.125	0.5
BT1 706	0.06	0.125	1	113 6304	0.06	0.125	1
BV1 1183	0.125	0.25	1	114 386	0.06	0.125	4
BW1 3130	0.125	0.125	1	115 5985	0.015	0.25	2
BX1 4271	0.125	0.25	1	116 5702	0.06	0.125	1
CN1 667	0.25	0.25	1	117 6026	0.06	0.125	2
CB1 1584	0.25	0.125	1	120 6057	0.03	0.25	1
CF1 5922	0.125	0.125	1	121 6072	0.06	0.25	0.5
CG1 1566	0.125	0.125	1	122 6111	0.25	0.25	0.5
CL1 3851	0.25	0.125	1	100 6353	0.125	0.25	1

Example 6

Activity of OPT-80 Compared Against Selected Anaerobic Species

The in vitro activity of OPT-80 was determined against 350 anaerobes. The experimental procedure for which is outlined in Antimicrobial Agents and Chemotherapy, 2004, 48: 4430-4434, which is hereby incorporated by reference in its entirety.

All organisms, including the 21 *C. difficile* strains, were separate isolates and not clonally related. All quality-control gram-negative and -positive strains recommended by NCCLS were included with each run: in every case, results (where available) were in range.

Results of MIC testing are presented in Table 7.

TABLE 7

MICs (μg/ml) of R-Tiacumicin B (>90% Stereomerically Pure)

Organism	MIC range	MIC_{50}	MIC_{90}
Bacteroides fragilis (19)	64->128	>128	>128
Non-fragilis B. fragilis group species (38)	64->128	>128	>128
Prevotella/Porphyromonas species (42)	16->128	>128	>128
Fusobacterium nucleatum (14)	64->128	>128	>128
Fusobacterium mortiferum (10)	64->128	>128	>128
Fusobacterium species, miscellaneous (14)	16->128	>128	>128
Peptostreptococcus tetradius (16)	0.25-2.0	1.0	1.0
Peptostreptococcus asaccharolyticus (15)	0.25-1.0	0.5	1.0
Peptostreptococcus anaerobius (15)	<0.016-0.03	<0.016	<0.016
Finegoldia magna (15)	0.25-2.0	1.0	1.0
Micromonas micros (14)	<0.016-0.06	0.03	0.06
Peptostreptococcus prevotii (3)	0.25-1.0	NA	NA
Propionibacterium acnes (20)	0.5-1.0	4.0	4.0
Eggerthella lenta (10)	<0.016-0.06	<0.016	<0.03
Miscellaneous gram-positive non-spore-forming rods (20)	<0.016-16	<0.125	16
Clostridium perfringens (35)	<0.016-0.06	<0.016	0.03
Clostridium difficile (21)	<0.016-0.25	<0.016	0.125
Clostridium tertium (10)	<0.016-0.06	<0.016	0.03
Clostridium species (19)	<0.016-0.06	<0.016	0.03
Clostridium spp. (all) (85)	<0.016-0.06	<0.016	0.06

TABLE 7-continued

MICs (μg/ml) of R-Tiacumicin B (>90% Stereomerically Pure)

Example 7

In Vitro Activities of OPT-80 Against Intestinal Bacteria

The in vitro activity of OPT-80 against intestinal bacteria was evaluated. The experimental procedure for which is outlined in Antimicrobial Agents and Chemotherapy, 2004, 48: 4898-4902, which is hereby incorporated by reference in its entirety.

Antimicrobial concentration ranges were selected to encompass or surpass the levels that would be achieved in the gut (to the extent that this information is available), subject to the limitations of solubility of the drugs in the testing medium. The range of concentration of OPT-80 used during testing was 0.03 μg/ml to 1024 μg/ml.

For analysis, the bacteria tested were generally placed into genus, species, or other groups with at least 10 isolates. The ranges and the MICs at which 50 and 90% of isolates were inhibited were determined except for organisms with fewer than 10 strains tested, for which only the ranges are reported (Table 8).

OPT-80 had good activity against most anaerobic gram-positive non-spore-forming rods and anaerobic gram-positive cocci. OPT-80 also showed good activity against enterococci and staphylococci.

TABLE 8

In vitro activity of R-Tiacumicin B (>90% Stereomerically Pure) against 453 bacterial isolates

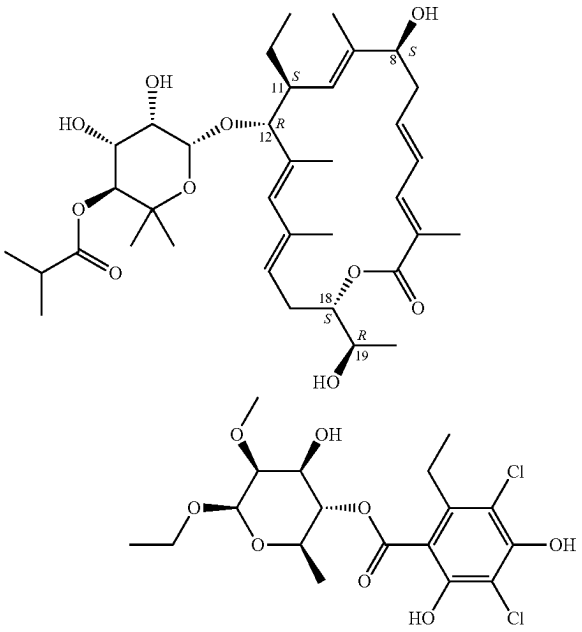
Organism	MIC range	MIC_{50}	MIC_{90}
Bacteroides fragilis group spp. (50)	256->1024	256	>1024
Veillonella spp. (10)	16-128	32	128
Other anaerobic gram-negative rods (51)	0.06-1024	1024	>1024
All anaerobic gram-negative species (111)	0.06->1024	256	>1024
Clostridium bifermentans (9)	0.06	NA	NA
Clostridium bolteae (7)	1-64	NA	NA
Clostridium clostridioforme (4)	4-128	NA	NA
Clostridium difficile (23)	0.06-2	0.12	0.25
Clostridium glycolicum (9)	0.06-1	NA	NA
Clostridium innocuum (9)	32-128	NA	NA
Clostridium paraputrificum (8)	0.06-8	NA	NA
Clostridium perfringens (14)	0.06	0.062	0.062
Clostridium ramosum (10)	256-512	512	512
Clostridium sordellii (5)	0.06	NA	NA
Other clostridial species (9)	0.06->1024	NA	NA
All *Clostridium* species (107)	0.06->1024	0.062	128
Anaerobic non-spore-forming gram-positive rods (63)	0.06->1024	1	32
Anaerobic gram-positive cocci (49)	0.06->1024	0.5	2
All anaerobic gram-positive species (219)	0.06->1024	0.12	64
Streptococcus, formerly *S. milleri* group (14)	16-64	32	32
Other *Streptococcus* species (9)	16-128	NA	NA
Enterococcus species (21)	2.0-16	8	8
Staphylococcus aureus and *Staphylococcus epidermidis* (19)	0.25-2	0.5	2
Total for all strains (453)	0.06->1024	8	1024

Other Embodiments

All references discussed above are herein incorporated by reference in their entirety for all purposes. While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the appended claims.

What is claimed is:

1. An isolated compound having the formula:



free from other stereoisomers of the compound.

2. A pharmaceutical composition comprising the compound of claim **1** or pharmaceutically acceptable salt thereof.

3. The pharmaceutical composition of claim **2**, further comprising one or more pharmaceutically acceptable carriers.

4. The pharmaceutical composition of claim **3**, wherein the composition is formulated for oral administration.

5. The pharmaceutical composition of claim **4**, wherein the composition is formulated as a tablet.

6. A pharmaceutical composition consisting of the compound of claim **1** or pharmaceutically acceptable salt thereof and one or more pharmaceutically acceptable carriers.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

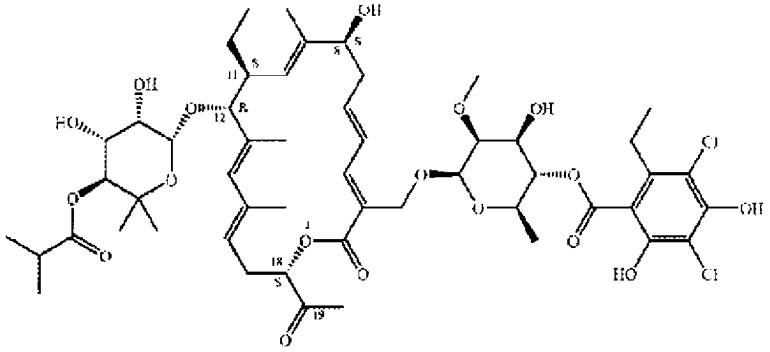
CERTIFICATE OF CORRECTION

PATENT NO. : 8,586,551 B2
APPLICATION NO. : 12/551056
DATED : November 19, 2013
INVENTOR(S) : Youe-Kong Shue et al.

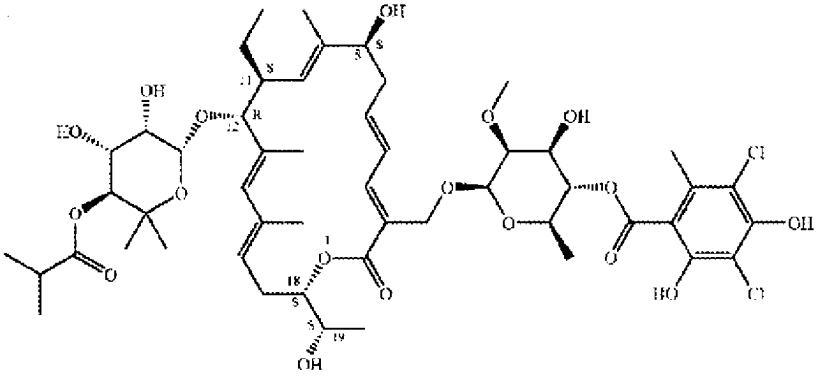
It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Specification

From column 7, line 49 to column 8, line 10, Formula II should appear as follows:



From column 8, line 50 to column 9, line 10, Formula III should appear as follows:

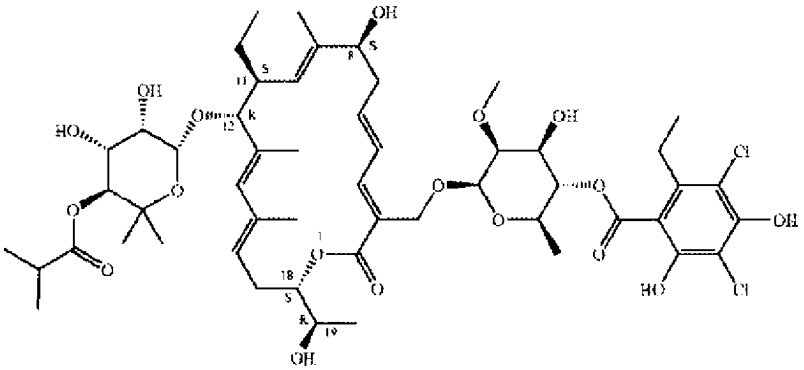


Signed and Sealed this
Eleventh Day of March, 2014

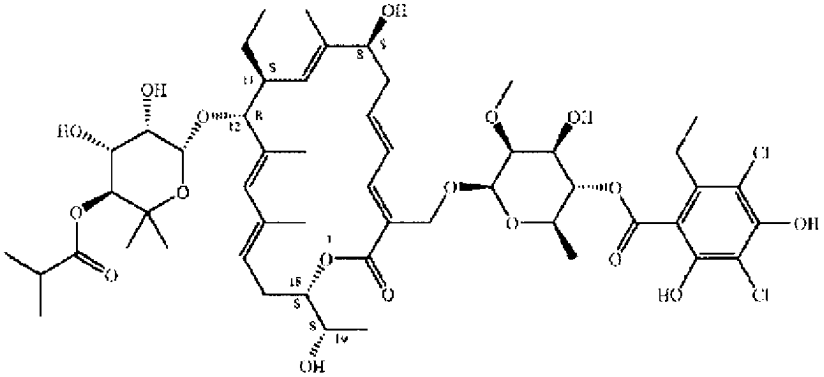
Michelle K. Lee

Michelle K. Lee
Deputy Director of the United States Patent and Trademark Office

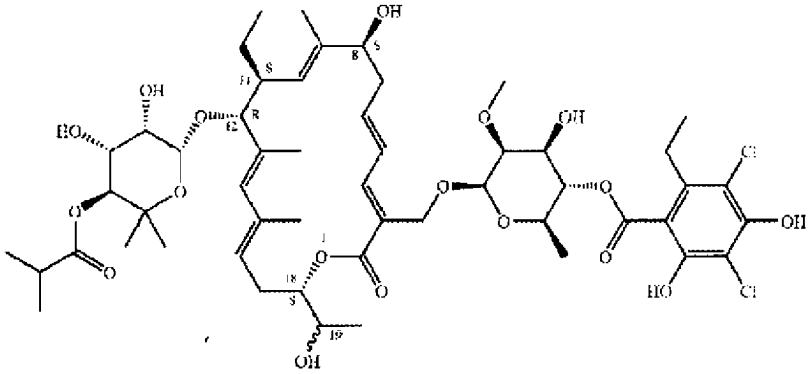
Column 11, lines 15-45, Formula IV should appear as follows:



From column 11, line 50 to column 12, line 10, Formula V should appear as follows:



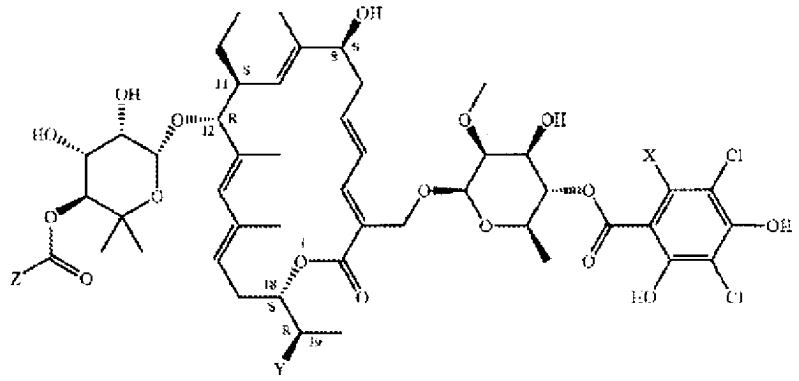
Column 13, lines 3-28, Formula VI should appear as follows:



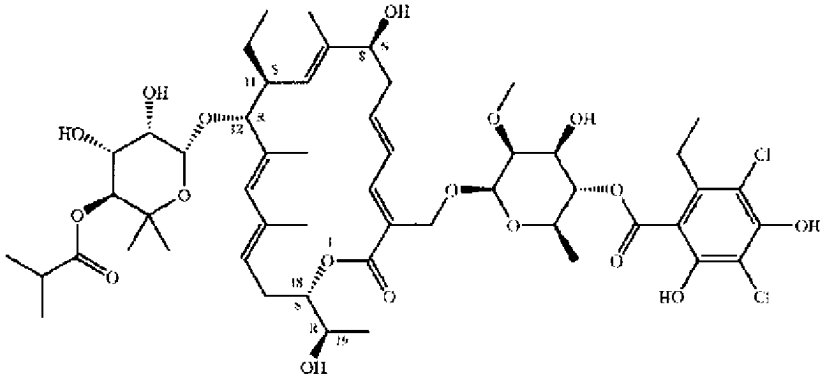
CERTIFICATE OF CORRECTION (continued)

U.S. Pat. No. 8,586,551 B2

From column 13, line 51 to column 14, line 10, Formula VII should appear as follows:



Column 18, lines 50-55, Formula IV should appear as follows:



In the Claims

In claim 1 (column 30, lines 8-33), Formula IV should appear as follows:

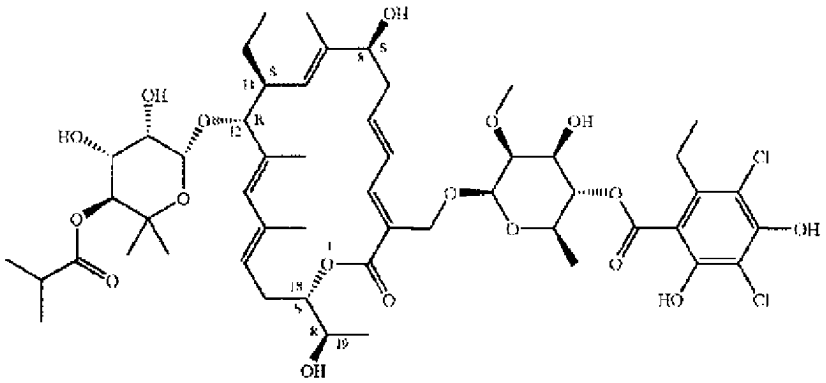


EXHIBIT 3

US007378508B2

(12) **United States Patent**
Chiu et al.

(10) **Patent No.: US 7,378,508 B2**
(45) **Date of Patent: May 27, 2008**

(54) **POLYMORPHIC CRYSTALLINE FORMS OF TIACUMICIN B**

(75) Inventors: **Yu-Hung Chiu**, San Diego, CA (US); **Tessie Mary Che**, San Diego, CA (US); **Alex Romero**, San Diego, CA (US); **Yoshi Ichikawa**, San Diego, CA (US); **Youe-Kong Shue**, Carlsbad, CA (US)

(73) Assignee: **Optimer Pharmaceuticals, Inc.**, San Diego, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **11/831,886**

(22) Filed: **Jul. 31, 2007**

(65) **Prior Publication Data**

US 2007/0259949 A1 Nov. 8, 2007

Related U.S. Application Data

(63) Continuation-in-part of application No. PCT/US2005/002887, filed on Jan. 31, 2005.

(60) Provisional application No. 60/881,950, filed on Jan. 22, 2007.

(51) **Int. Cl.**
C07H 17/08 (2006.01)
C07D 313/00 (2006.01)

(52) **U.S. Cl.** **536/7.1**; 549/271

(58) **Field of Classification Search** ..... None
See application file for complete search history.

(56) **References Cited**

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J.E. Hochlowski et al., Tiacumicins, A Novel Complex of 18-Membered Macrolides, *J. Antibiotics*, vol. XL, No. 5, pp. 575-588 (May 1987).

* cited by examiner

Primary Examiner—Shaojia Anna Jiang
Assistant Examiner—Eric S Olson
(74) *Attorney, Agent, or Firm*—Morgan Lewis & Bockius LLP

(57) **ABSTRACT**

The invention relates to novel forms of compounds displaying broad spectrum antibiotic activity, especially crystalline polymorphic forms and amorphous forms of such compounds, compositions comprising such crystalline polymorphic forms and amorphous forms of such compounds, processes for manufacture and use thereof. The compounds and compositions of the invention are useful in the pharmaceutical industry, for example, in the treatment or prevention of diseases or disorders associated with the use of antibiotics, chemotherapies, or antiviral therapies, including, but not limited to, colitis, for example, pseudo-membranous colitis; antibiotic associated diarrhea; and infections due to *Clostridium difficile* ("*C. difficile*"), *Clostridium perfringens* ("*C. perfringens*"), *Staphylococcus* species, for example, methicillin-resistant *Staphylococcus*, or *Enterococcus* including Vancomycin-resistant *enterococci.*

20 Claims, 3 Drawing Sheets

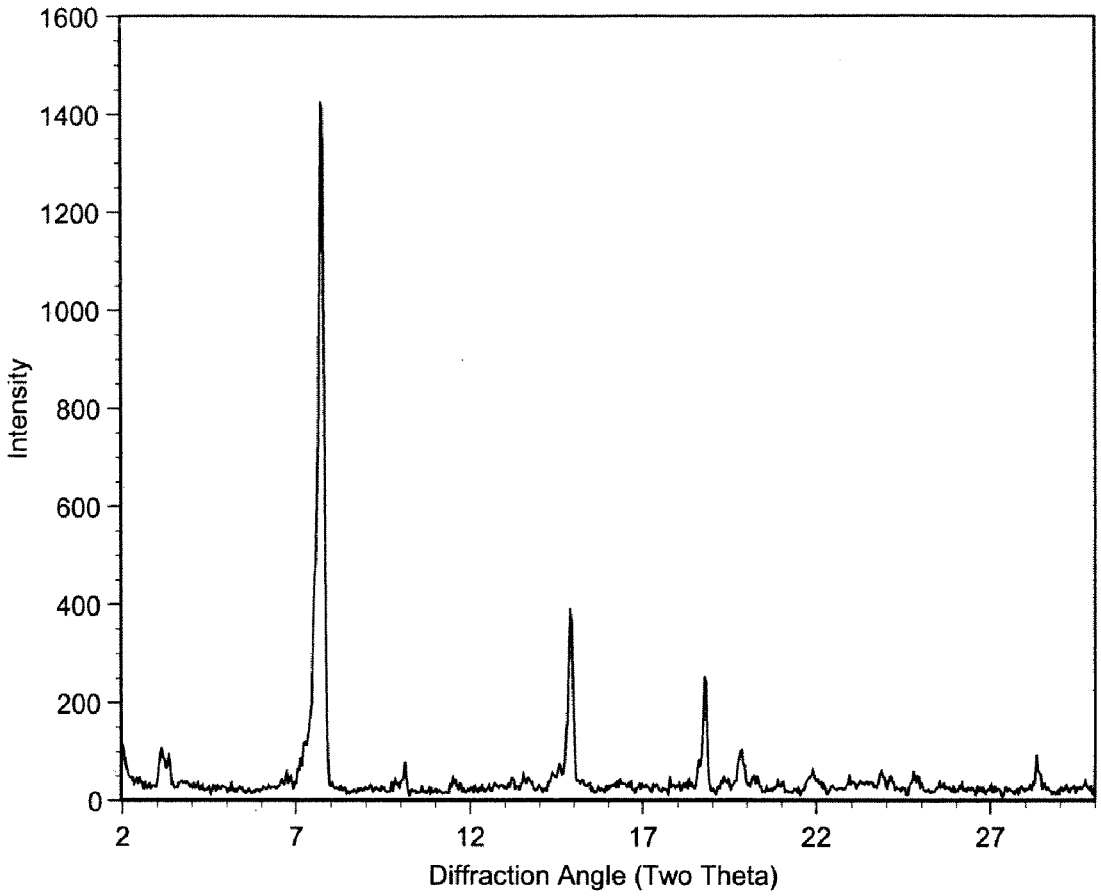

1600
1400
1200
1000
800
600
400
200
0
Intensity
2
7
12
17
22
27
Diffraction Angle (Two Theta)

Figure 1

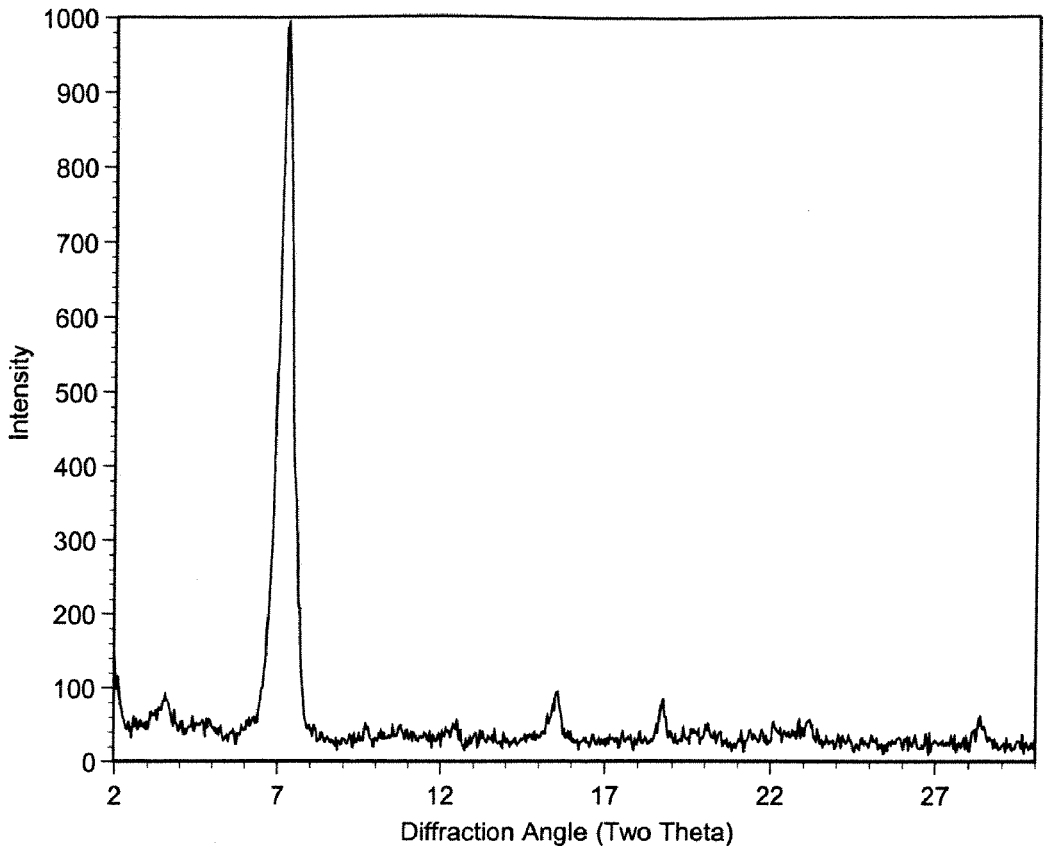

1000
900
800
700
600
500
400
300
200
100
0
Intensity
2
7
12
17
22
27
Diffraction Angle (Two Theta)

Figure 2

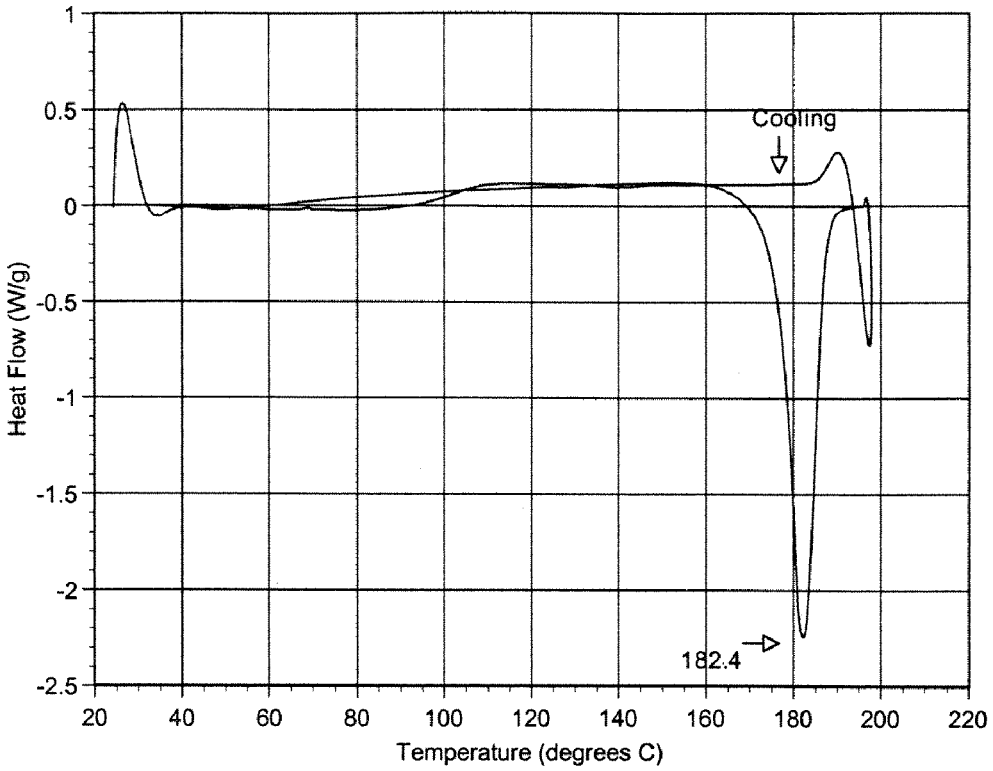

1
0.5
0
-0.5
-1
-1.5
-2
-2.5
Heat Flow (W/g)
Cooling
182.4
20
40
60
80
100
120
140
160
180
200
220
Temperature (degrees C)

Figure 3

POLYMORPHIC CRYSTALLINE FORMS OF TIACUMICIN B

1. RELATED APPLICATIONS

The present application is a continuation-in-part application of PCT Application PCT/US05/02887, filed Jan. 31, 2005, and claims the benefit of U.S. provisional patent application No. 60/881,950, filed Jan. 22, 2007, the entire disclosures of each are herein incorporated by reference.

2. FIELD OF THE INVENTION

The invention encompasses novel forms of compounds displaying broad spectrum antibiotic activity, especially crystalline polymorphic forms and amorphous forms of such compounds, compositions comprising such crystalline polymorphic forms and amorphous forms of such compounds, processes for manufacture and use thereof. The compounds and compositions of the invention are useful in the medical and pharmaceutical industry, for example, in the treatment or prevention of diseases or disorders associated with the use of antibiotics, chemotherapies, or antiviral therapies, including, but not limited to, colitis, for example, pseudo-membranous colitis; antibiotic associated diarrhea; and infections due to *Clostridium difficile* ("*C. difficile*"), *Clostridium perfringens* ("*C. perfringens*"), *Staphylococcus* species, for example, methicillin-resistant *Staphylococcus*, or *Enterococcus* including Vancomycin-resistant *enterococci*.

3. BACKGROUND OF THE INVENTION

Antibiotic-associated diarrhea ("AAD") diseases are caused by toxin producing strains of *C. difficile, Staphylococcus aureus* ("*S. aureus*") including methicillin-resistant *Staphylococcus aureus* ("MRSA") and *C. perfringens*. AAD represents a major economic burden to the healthcare system that is conservatively estimated at $3-6 billion per year in excess hospital costs in the United States alone.

AAD is a significant problem in hospitals and long-term care facilities. *C. difficile* is the leading cause of AAD in the hospital setting, accounting for approximately 20% of cases of AAD and the majority of cases of antibiotic-associated colitis ("AAC"). The rising incidence of *C. difficile* associated diarrhea ("CDAD") has been attributed to the frequent prescribing of broad-spectrum antibiotics to hospitalized patients.

The tiacumicins are a group of 18-membered macrolide antibiotics originally isolated from the fermentation broth of *Dactylosporangium aurantiacum*. The tiacumicins are effective Gram-positive antibiotics. In particular, tiacumicins, specifically Tiacumicin B, show activity against a variety of bacterial pathogens and in particular against *C. difficile*, a Gram-positive bacterium (*Antimicrob. Agents Chemother.*, 1991, 1108-1111). A purification of tiacumicins was carried out in suitable solvents, wherein tiacumicin B exhibited a melting point of 143-145° C. (See, e.g., J. E. Hochlowski, et al., *J. Antibiotics*, vol. XL, no. 5, pages 575-588 (1987)).

The polymorphic behavior of a compound can be of crucial importance in pharmacy and pharmacology. Polymorphs are, by definition, crystals of the same molecule having different physical properties as a result of the order

of the molecules in the crystal lattice. The differences in physical properties exhibited by polymorphs affect pharmaceutical parameters such as storage stability, compressibility and density (important in formulation and product manufacturing), and dissolution rates (an important factor in determining bio-availability). Differences in stability can result from changes in chemical reactivity (e.g., differential oxidation, such that a dosage form discolors more rapidly when comprised of one polymorph than when comprised of another polymorph) or mechanical changes (e.g., tablets crumble on storage as a kinetically favored polymorph converts to thermodynamically more stable polymorph) or both (e.g., tablets of one polymorph are more susceptible to breakdown at high humidity). As a result of solubility/dissolution differences, in the extreme case, some polymorphic transitions may result in lack of potency or, at the other extreme, toxicity. In addition, the physical properties of a crystal may be important in processing: for example, one polymorph might be more likely to form solvates or might be difficult to filter and wash free of impurities (i.e., particle shape and size distribution might be different between one polymorph relative to the other).

Each pharmaceutical compound has an optimal therapeutic blood concentration and a lethal concentration. The bio-availability of the compound determines the dosage strength in the drug formulation necessary to obtain the ideal blood level. If the drug can crystallize as two or more polymorphs differing in bio-availability, the optimal dose will depend on the polymorph present in the formulation. Some drugs show a narrow margin between therapeutic and lethal concentrations. Thus, it becomes important for both medical and commercial reasons to produce and market the drug in its most thermodynamically stable polymorph, substantially free of other kinetically favored or disfavored polymorphs.

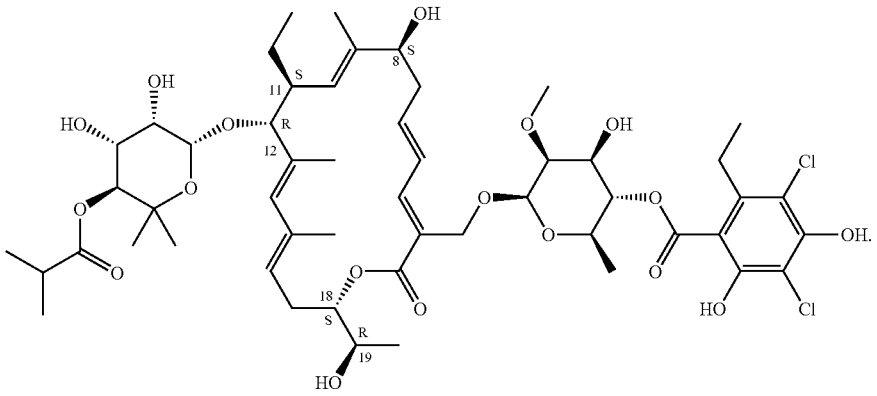
Thus, there is a clear need to develop safe and effective polymorphs of drugs that are efficacious at treating or preventing disorders associated with bacterial pathogens. The present inventors have identified novel crystalline and amorphous forms of 18-membered macrolide compounds that exhibit broad spectrum antibiotic activity.

4. SUMMARY OF THE INVENTION

The invention encompasses novel crystalline and amorphous forms of the macrolide compounds that are useful in treating or preventing bacterial infections and protozoal infections. In an illustrative embodiment, the novel crystalline and amorphous forms of the macrolide compounds of the invention exhibit broad spectrum antibiotic activity. Thus, surprisingly novel crystalline and amorphous forms of the macrolide compounds have been identified, which act as antibiotics possessing a broad spectrum of activity in treating or preventing bacterial infections and protozoal infections, especially those associated with Gram-positive and Gram-negative bacteria and in particular, Gram-positive bacteria.

In one embodiment, the invention encompasses novel crystalline and amorphous forms of the macrolide of Formula I:

Formula I



In another embodiment, the invention encompasses a mixture of compounds with varying amounts of the Compound of Formula I, which forms have the requisite stability for use in preparing pharmaceutical compositions.

In another embodiment, the invention encompasses a polymorph obtained from a mixture of tiacumicins and a Compound of Formula I.

In still another embodiment, the invention encompasses novel crystalline and amorphous forms of the Compound of Formula I.

In another embodiment, the invention encompasses a pharmaceutical composition comprising a Compound of Formula I.

In another embodiment, the invention encompasses a pharmaceutical composition comprising a Compound of Formula I, wherein the Compound of Formula I is present in an amount greater than 90% by weight.

In another embodiment, the invention encompasses a pharmaceutical composition comprising one or more novel crystalline and amorphous forms of a Compound of Formula I.

In another embodiment, the invention encompasses a pharmaceutical composition comprising a mixture of tiacumicins and Compound of Formula I.

In another embodiment, the invention encompasses a pharmaceutical composition comprising a mixture of tiacumicins and at least about 75% or more by weight of Compound of Formula I. In another embodiment, the invention encompasses a pharmaceutical composition comprising a mixture of tiacumicins and at least about 80% or more by weight of Compound of Formula I. In another embodiment, the invention encompasses a pharmaceutical composition comprising a mixture of tiacumicins and at least about 85% or more by weight of Compound of Formula I. In another embodiment, the invention encompasses a pharmaceutical composition comprising a mixture of tiacumicins and at least about 90% or more by weight of Compound of Formula I. In another embodiment, the invention encompasses a pharmaceutical composition comprising a mixture of tiacumicins and at least about 95% or more by weight of Compound of Formula I. In another embodiment, the invention encompasses a pharmaceutical composition comprising a mixture of tiacumicins and at least about 99% or more by weight of Compound of Formula I.

The invention also encompasses methods for treating or preventing a disease or disorder including, but not limited to, bacterial infections and protozoal infections comprising administering to a subject, preferably a mammal, in need thereof a therapeutically or prophylactically effective amount of a composition or formulation comprising a compound of the invention.

In one illustrative embodiment, the composition or formulation comprises a mixture of compounds with varying amounts of the Compound of Formula I. In another embodiment, the composition or formulation comprises a mixture of tiacumicins and a Compound of Formula I. In still another embodiment, the composition or formulation comprises novel crystalline and amorphous forms of the Compound of Formula I. In still another embodiment, the composition or formulation comprises novel crystalline and amorphous forms of the Compound of Formula I and a mixture of tiacumicins.

In another particular embodiment, the disease or disorder to be treated or prevented are caused by toxin producing strains of *C. difficile, Staphylococcus aureus* ("*S. aureus*") including methicillin-resistant *Staphylococcus aureus* ("MRSA") and *C. perfringens*. In another particular embodiment, the disease or disorder to be treated or prevented is antibiotic-associated diarrhea.

5. BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** shows the X-ray powder diffraction patterns of a first polymorph Compound of Formula I produced from methanol and water.

FIG. **2** shows the X-ray powder diffraction patterns of a second polymorph Compound of Formula I produced from ethyl acetate.

FIG. **3** shows the effect of temperature on a mixture of tiacumicins produced from methanol and water. The DSC indicates an endothermic curve beginning at 169° C., and weight loss beginning at 223° C. The endothermic curve at about 177° C. corresponds to the melting of a first polymorph of a Compound of Formula I.

6. DETAILED DESCRIPTION OF THE DRAWINGS

6.1. General Description

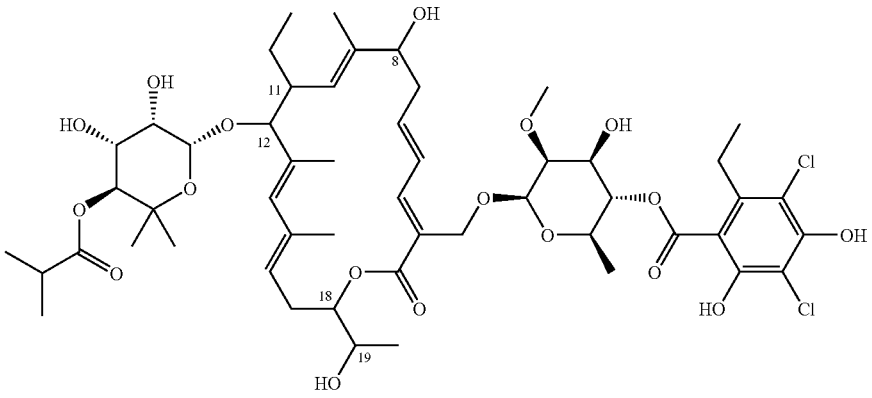
The invention broadly encompasses mixtures of compounds with varying amounts of the Compound of Formula I. The inventors have surprisingly determined that the formation of crystalline polymorphic forms and amorphous forms of a Compound of Formula I and optionally mixtures of tiacumicin depends on the selection of the crystallization solvent and on the method and conditions of crystallization or precipitation.

In one embodiment the invention encompasses a mixture of tiacumicins and a Compound of Formula I. In another embodiment, the invention encompasses novel crystalline and amorphous forms of the Compound of Formula I and optionally a mixture of tiacumicins. In still another embodiment, the invention encompasses novel crystalline and amorphous forms of the Compound of Formula I and a mixture of tiacumicins. In another embodiment, the invention encompasses a mixture of comprising a first polymorph of a Compound of Formula I, a second polymorph of a Compound of Formula I, and other polymorphic forms, amorphous forms and mixtures thereof.

In another particular embodiment, the crystalline polymorphs and amorphous forms are obtained from a mixture of tiacumicins.

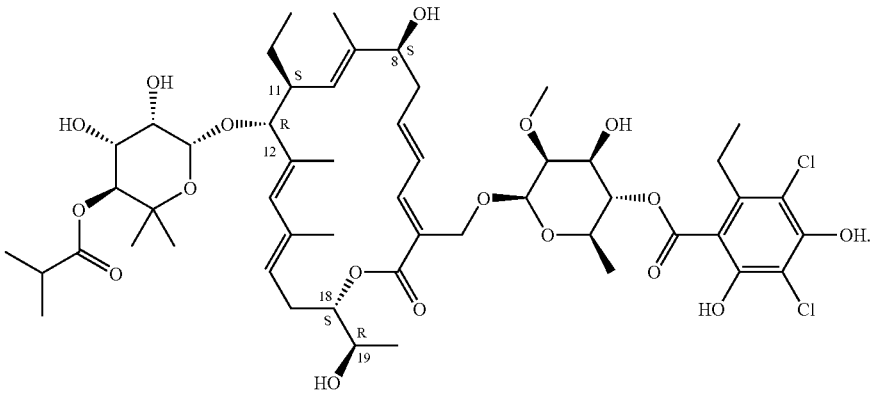
In another embodiment, a crystalline polymorph of a Compound of Formula I exhibits a representative powder diffraction pattern comprising at least peaks at the following diffraction angles 2θ of 7.7°, 15.0°, and 18.8°±0.04, preferably ±0.1, more preferably ±0.15, even more preferably ±0.2. In another embodiment, a crystalline polymorph of a Compound of Formula I exhibits a representative powder diffraction pattern comprising at least peaks at the following diffraction angles 2θ of 7.8°, 15.1°, and 18.8°±0.04, preferably ±0.1, more preferably ±0.15, even more preferably ±0.2.

In another embodiment, the polymorph has the chemical structure:



In another embodiment, the polymorph has the chemical structure of a Compound of Formula I:

Formula I



In another embodiment, the polymorph further comprises at least one compound selected from a mixture of tiacumicins.

In another embodiment, the polymorph of Formula I is present in an amount from at least about 75% to about 99.99%.

In another embodiment, the polymorph of Formula I is present in an amount of at least about 75%.

In another embodiment, the polymorph of Formula I is present in an amount of at least about 80%.

In another embodiment, the polymorph of Formula I is present in an amount of at least about 85%.

In another embodiment, the polymorph of Formula I is present in an amount of at least about 90%.

In another embodiment, the polymorph of Formula I is present in an amount of at least about 93%.

In another embodiment, the polymorph of Formula I is present in an amount of at least about 95%.

In another embodiment, the polymorph of Formula is present in an amount of at least about 99%.

In another embodiment, the crystalline polymorph is obtained from a mixture of tiacumicins that exhibits a melting point of about 163° C. to about 169° C. In another embodiment, the crystalline polymorph is obtained from a mixture of tiacumicins that exhibits a melting point of about 160° C. to about 170° C. In another embodiment, the crystalline polymorph is obtained from a mixture of tiacumicins that exhibits a melting point of about 155° C. to about 175° C.

In another embodiment, the crystalline polymorph is obtained from a mixture of tiacumicins and exhibits a DSC endotherm in the range of about 174° C. to about 186° C.; preferably 175-185° C.

In another embodiment, the crystalline polymorph is obtained from a mixture of tiacumicins that exhibits a powder diffraction pattern comprising at least peaks at the following diffraction angles 2θ of 7.7°, 15.0°, and 18.8°±0.04, preferably ±0.1, more preferably ±0.15, even more preferably ±0.2 and exhibits a melting point of about 163° C. to about 169° C.

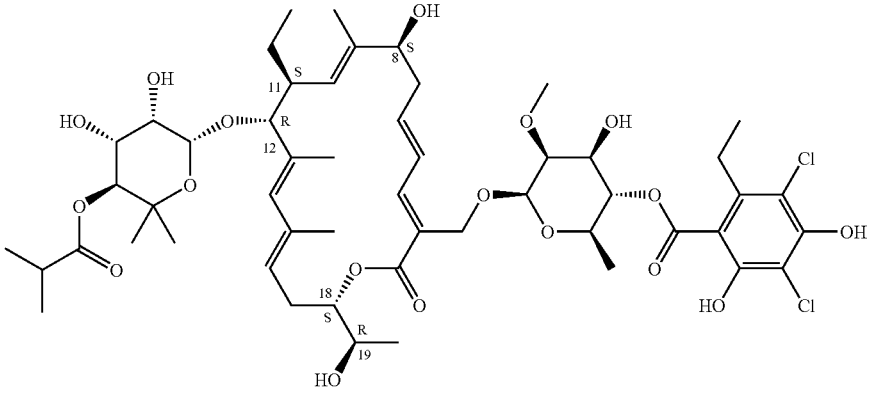
In another embodiment, the crystalline polymorph is obtained from a mixture of tiacumicins that exhibits a powder diffraction pattern comprising at least peaks at the following diffraction angles 2θ of 7.7°, 15.0°, and 18.8°±0.04, preferably ±0.1, more preferably ±0.15, even more preferably ±0.2 and exhibits a melting point of about 160° C. to about 170° C.

Another embodiment encompasses a crystalline polymorph obtained from a mixture of tiacumicins that exhibits a powder diffraction pattern comprising at least peaks at the following diffraction angles 2θ of 7.7, 15.0°, and 18.8°±0.04, preferably ±0.1, more preferably ±0.15, even more preferably ±0.2. In a particular embodiment, the polymorph has the chemical structure of a Compound of Formula I. In another embodiment, the crystalline polymorph further comprises at least one compound selected from a mixture of tiacumicins.

In another embodiment, a crystalline polymorph is obtained from a mixture of tiacumicins that exhibits a melting point of about 150° C. to about 156° C.

In another embodiment, a crystalline polymorph is obtained from a mixture of tiacumicins that exhibits a powder diffraction pattern comprising at least peaks at the following diffraction angles 2θ of 7.4°, 15.5°, and 18.8°±0.2 and exhibits a melting point of about 150° C. to about 156° C.

Another embodiment of the invention encompasses pharmaceutical compositions comprising a therapeutically or prophylactically effective amount of a crystalline polymorph of a Compound of Formula:



and a pharmaceutically acceptable carrier.

In a particular embodiment, the pharmaceutical composition comprises a first polymorph of a Compound of Formula I, a second polymorph of a Compound of Formula I, other polymorphic forms of a Compound of Formula I, amorphous forms of a Compound of Formula I, and mixtures thereof.

In another embodiment, the crystalline polymorph of the pharmaceutical composition has peaks at the following diffraction angles 2θ of 7.7°, 15.0°, and 18.8°±0.04, preferably ±0.1, more preferably ±0.15, even more preferably ±0.2.

In another embodiment, the crystalline polymorph of the pharmaceutical composition further comprises at least one compound selected from a mixture of tiacumicins.

In another embodiment, the Compound of Formula I is present from at least about 75% to about 99.99%, preferably about 75%, about 85%, about 95%, or about 99%.

In another embodiment, the crystalline polymorph of the pharmaceutical composition exhibits a melting point of about 163° C. to about 169° C.

Another embodiment encompasses a pharmaceutical composition comprising a crystalline polymorph of tiacumi-

cin comprising peaks at the following diffraction angles 2θ of 7.6°, 15.4°, and 18.8°±0.04, preferably ±0.1, more preferably ±0.15, even more preferably ±0.2. In a particular embodiment, the pharmaceutical composition further comprises at least one compound selected from a mixture of tiacumicins. In another particular embodiment, the Compound of Formula I is present from about 75% to about 99.99%, preferably 75%, 85%, 95%, or 99%.

In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 15% of a mixture of tiacumicins. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 10% of a mixture of tiacumicins. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 7% of a mixture of tiacumicins. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 5% of a mixture of tiacumicins. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 1% of a mixture of tiacumicins. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 15% of a mixture of S-Tiacumicin. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 10% of a mixture of S-Tiacumicin. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 7% of a mixture of S-Tiacumicin. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 5% of a mixture of S-Tiacumicin. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 1% of a mixture of S-Tiacumicin. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 15% of a mixture of Lipiarmycin A4. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 10% of a mixture of Lipiarmycin A4. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 7% of a mixture of Lipiarmycin A4. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 5% of a mixture of Lipiarmycin A4. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 1% of a mixture of Lipiarmycin A4.

In another embodiment, the crystalline polymorph of the pharmaceutical composition exhibits a melting point of about 153° C. to about 156° C.

In another embodiment, the therapeutically or prophylactically effective amount is from about 0.01 mg/kg to about 1000 mg/kg, preferably 0.01, 0.1, 1, 2.5, 5, 10, 20, 50, 100, 250, or 500 mg/kg.

In another embodiment, the crystalline polymorph of the pharmaceutical composition is suitable for parenteral administration, preferably intravenous, intramuscular, or intraarterial.

In another embodiment, the crystalline polymorph of the pharmaceutical composition is suitable for peroral administration.

Another embodiment of the invention encompasses a method for treating a bacterial infection comprising administering a pharmaceutical composition comprising a polymorph of the invention to a subject in need thereof.

In a particular embodiment, the bacterial infection is in the gastrointestinal tract, particularly AAC or AAD.

6.2. Definitions

The term "antibiotic-associated condition" refers to a condition resulting when antibiotic therapy disturbs the balance of the microbial flora of the gut, allowing pathogenic organisms such as enterotoxin producing strains of *C. difficile, S. aureus* and *C. perfringens* to flourish. These organisms can cause diarrhea, pseudomembranous colitis, and colitis and are manifested by diarrhea, urgency, abdominal cramps, tenesmus, and fever among other symptoms. Diarrhea, when severe, causes dehydration and the medical complications associated with dehydration.

The term "asymmetrically substituted" refers to a molecular structure in which an atom having four tetrahedral valences is attached to four different atoms or groups. The commonest cases involve the carbon atom. In such cases, two optical isomers (D- and L-enantiomers or R- and S-enantiomers) per carbon atom result which are nonsuperposable mirror images of each other. Many compounds have more than one asymmetric carbon. This results in the possibility of many optical isomers, the number being determined by the formula 2n, where n is the number of asymmetric carbons.

The term "broth" as used herein refers to the fluid culture medium as obtained during or after fermentation. Broth comprises a mixture of water, the desired antibiotic(s), unused nutrients, living or dead organisms, metabolic products, and the adsorbent with or without adsorbed product.

As used herein and unless otherwise indicated, the terms "bacterial infection(s)" and "protozoal infection(s)" are used interchangeably and include bacterial infections and protozoal infections that occur in mammals, fish and birds as well as disorders related to bacterial infections and protozoal infections that may be treated or prevented by antibiotics such as the Compounds of the Invention. Such bacterial infections and protozoal infections, and disorders related to such infections, include the following: disorders associated with the use of antibiotics, chemotherapies, or antiviral therapies, including, but not limited to, colitis, for example, pseudo-membranous colitis, antibiotic associated diarrhea, and infections due to *Clostridium difficile, Clostridium perfringens, Staphylococcus* species, methicillin-resistant *Staphylococcus*, or *Enterococcus* including Vancomycin-resistant *enterococci*; antibiotic-associated diarrhea including those caused by toxin producing strains of *C. difficile, S. aureus* including methicillin-resistant *Staphylococcus aureus*, and *C. perfringens*; and antibiotic-associated colitis; pneumonia, otitis media, sinusitis, bronchitis, tonsillitis and mastoiditis related to infection by *Staphylococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Staphlococcus aureus*, or *Peptostreptococcus* spp.; pharynigis, rheumatic fever and glomerulonephritis related to infection by *Streptococcus pyogenes*, Groups C and G *streptococci, Clostridium diptheriae*, or *Actinobacillus haemolyticum*; respiratory tract infections related to infection by *Mycoplasma pneumoniae, Legionella pneumophila, Streptococcus pneumoniae, Haemophilus influenzae*, or *Chlamydia pneumoniae*; uncomplicated skin and soft tissue infections, abscesses and osteomyelitis, and puerperal fever related to infection by *Staphlococcus aureus*, coagulase-

positive *staphlococci* (e.g., *S. epidermis* and *S. hemolyticus*), *Staphylococcus pyogenes, Streptococcus agalactiae*, Streptococcal groups C-F (minute-colony *streptococci*), viridans streptococci, *Corynebacterium minutissimum, Clostridium* spp., or *Bartonella henselae*; uncomplicated acute urinary tract infections related to infection by *Staphylococcus saprophyticus* or *Enterococcus* spp.; urethritis and cervicitis; and sexually transmitted diseases related to infection by *Chlamydia trachomatis, Haemophilus ducreyi, Treponema pallidum, Ureaplasma urealyticum*, or Neiserria gonorrhea; toxin diseases related to infection by *S. aureus* (food poisoning and Toxic Shock Syndrome), or Groups A, B and C *streptococci*; ulcers related to infection by *Helicobacter pylori*, systemic febrile syndromes related to infection by *Borrelia recurrentis*; Lyme disease related to infection by *Borrelia burgdorferi*, conjunctivitis, keratitis, and dacrocystitis related to infection by *Chlamydia trachomatis, Neisseria gonorrhoeae, S. aureus, S. pneumoniae, S. pyogenes, H. influenzae*, or *Listeria* spp.; disseminated *Mycobacterium avium* complex (MAC) disease related to infection by *Mycobacterium avium*, or *Mycobacterium intracellulare*; gastroenteritis related to infection by *Campylobacter jejuni*, intestinal protozoa related to infection by *Cryptosporidium* spp.; odontogenic infection related to infection by viridans *streptococci*; persistent cough related to infection by *Bordetella pertussis*; gas gangrene related to infection by *Clostridium perfringens* or *Bacteroides* spp.; and atherosclerosis related to infection by *Helicobacter pylori* or *Chlamydia pneumoniae*. Bacterial infections and protozoal infections and disorders related to such infections that may be treated or prevented in animals include the following: bovine respiratory disease related to infection by *P. haem., P. multocida, Mycoplasma bovis*, or *Bordetella* spp.; cow enteric disease related to infection by *E. coli* or protozoa (e.g., coccidia, cryptosporidia, etc.); dairy cow mastitis related to infection by *Staph. aureus, Strep. uberis, Strep. agalactiae, Strep. dysgalactiae, Klebsiella* spp., *Corynebacterium*, or *Enterococcus* spp.; swine respiratory disease related to infection by *A. pleuro., P. multocida* or *Mycoplasma* spp.; swine enteric disease related to infection by *E. coli Lawsonia intracellularis*, Salmonella, or *Serpulina hyodyisinteriae*; cow footrot related to infection by *Fusobacterium* spp.; cow metritis related to infection by *E. coli*; cow hairy warts related to infection by *Fusobacterium necrophorum* or *Bacteroides nodosus*; cow pink-eye related to infection by *Moraxella bovis*; cow premature abortion related to infection by protozoa (e.g., neosporium) urinary tract infection in dogs and cats related to infection by *E. coli*; skin and soft tissue infections in dogs and cats related to infection by *Staph. epidermidis, Staph. intermedius*, coagulase neg. *Staph*. or *P. multocida*; and dental or mouth infections in dogs and cats related to infection by *Alcaligenes* spp., *Bacteroides* spp., *Clostridium* spp., *Enterobacter* spp., *Eubacterium, Peptostreptococcus, Porphyromonas*, or *Prevotella*. Other bacterial infections and protozoal infections and disorders related to such infections that may be treated or prevented in accord with the methods of the invention are referred to in Sanford, J. P., et al., "The Sanford Guide To Antimicrobial Therapy," 27th Edition (Antimicrobial Therapy, Inc., 1996).

As used herein and unless otherwise indicated, the term "binders" refers to agents used to impart cohesive qualities to the powdered material. Binders, or "granulators" as they are sometimes known, impart cohesiveness to the tablet formulation, which insures the tablet remaining intact after compression, as well as improving the free-flowing qualities by the formulation of granules of desired hardness and size. Materials commonly used as binders include starch; gelatin; sugars, such as sucrose, glucose, dextrose, molasses, and lactose; natural and synthetic gums, such as acacia, sodium alginate, extract of Irish moss, panwar gum, ghatti gum, mucilage of isapol husks, carboxymethylcellulose, methylcellulose, polyvinylpyrrolidone, Veegum, microcrystalline cellulose, microcrystalline dextrose, amylose, and larch arabogalactan, and the like.

As used herein and unless otherwise indicated, the terms "biohydrolyzable amide," "biohydrolyzable ester," "biohydrolyzable carbamate," "biohydrolyzable carbonate," "biohydrolyzable ureide," "biohydrolyzable phosphate" mean an amide, ester, carbamate, carbonate, ureide, or phosphate, respectively, of a compound that either: 1) does not interfere with the biological activity of the compound but can confer upon that compound advantageous properties in vivo, such as uptake, duration of action, or onset of action; or 2) is biologically inactive but is converted in vivo to the biologically active compound. Examples of biohydrolyzable esters include, but are not limited to, lower alkyl esters, lower acyloxyalkyl esters (such as acetoxylmethyl, acetoxyethyl, aminocarbonyloxy-methyl, pivaloyloxymethyl, and pivaloyloxyethyl esters), lactonyl esters (such as phthalidyl and thiophthalidyl esters), lower alkoxyacyloxyalkyl esters (such as methoxycarbonyloxy-methyl, ethoxycarbonyloxyethyl and isopropoxycarbonyloxyethyl esters), alkoxyalkyl esters, choline esters, and acylamino alkyl esters (such as acetamidomethyl esters). Examples of biohydrolyzable amides include, but are not limited to, lower alkyl amides, a amino acid amides, alkoxyacyl amides, and alkylaminoalkyl-carbonyl amides. Examples of biohydrolyzable carbamates include, but are not limited to, lower alkylamines, substituted ethylenediamines, aminoacids, hydroxyalkylamines, heterocyclic and heteroaromatic amines, and polyether amines.

As used herein and unless otherwise indicated, the term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which a composition is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like.

As used herein and unless otherwise indicated, the term "Compounds of the Invention" means, collectively, a Compound of Formula I and/or pharmaceutically acceptable salts and polymorphs thereof. The compounds of the invention are identified herein by their chemical structure and/or chemical name. Where a compound is referred to by both a chemical structure and a chemical name, and that chemical structure and chemical name conflict, the chemical structure is determinative of the compound's identity. The compounds of the invention may contain one or more chiral centers and/or double bonds and, therefore, exist as stereoisomers, such as double-bond isomers (i.e., geometric isomers), enantiomers, or diastereomers. According to the invention, the chemical structures depicted herein, and therefore the compounds of the invention, encompass all of the corresponding compound's enantiomers and stereoisomers, that is, both the stereomerically pure form (e.g., geometrically pure, enantiomerically pure, or diastereomerically pure) and enantiomeric and stereoisomeric mixtures. Enantiomeric and stereoisomeric mixtures can be resolved into their component enantiomers or stereoisomers by well known methods, such as chiral-phase gas chromatography, chiral-phase high performance liquid chromatography, crystallizing the compound as a chiral salt complex, or crystallizing the compound in a chiral solvent. Enantiomers and stereoisomers can also be obtained from stereomerically- or enantiomerically-pure intermediates, reagents, and catalysts by well known asymmetric synthetic methods. The Compounds of the Invention are preferably substantially stereomerically

pure. In a particular embodiment, the term "Compounds of the Invention" refers to a Compound of Formula that is greater than 75% pure, preferably greater than 85% pure, more preferably greater than 95% pure and most preferably greater than 99% pure and polymorphic form (e.g., a polymorph of Compound of Formula I) and amorphous forms thereof.

As used herein and unless otherwise indicated, "diluents" are inert substances added to increase the bulk of the formulation to make the tablet a practical size for compression. Commonly used diluents include calcium phosphate, calcium sulfate, lactose, kaolin, mannitol, sodium chloride, dry starch, powdered sugar, silica, and the like.

As used herein and unless otherwise indicated, "disintegrators" or "disintegrants" are substances that facilitate the breakup or disintegration of tablets after administration. Materials serving as disintegrants have been chemically classified as starches, clays, celluloses, algins, or gums. Other disintegrators include Veegum HV, methylcellulose, agar, bentonite, cellulose and wood products, natural sponge, cation-exchange resins, alginic acid, guar gum, citrus pulp, cross-linked polyvinylpyrrolidone, carboxymethylcellulose, and the like.

When administered to a subject (e.g., to an animal for veterinary use or to a human for clinical use) the compounds of the invention are administered in isolated form. As used herein and unless otherwise indicated, "isolated" means that the compounds of the invention are separated from other components of either (a) a natural source, such as a plant or cell, preferably bacterial culture, or (b) a synthetic organic chemical reaction mixture, preferably, via conventional techniques, the compounds of the invention are purified. As used herein, "purified" means that when isolated, the isolate contains at least about 70% preferably at least about 80%, more preferably at least about 90%, even more preferably at least about 95%, and most preferably at least about 99% of a compound of the invention by weight of the isolate.

The term "macrolide" or "macrocycle" refers to organic molecules with large ring structures usually containing over 10 atoms.

The term "18-membered macrocycles" refers to organic molecules with ring structures containing 18 atoms.

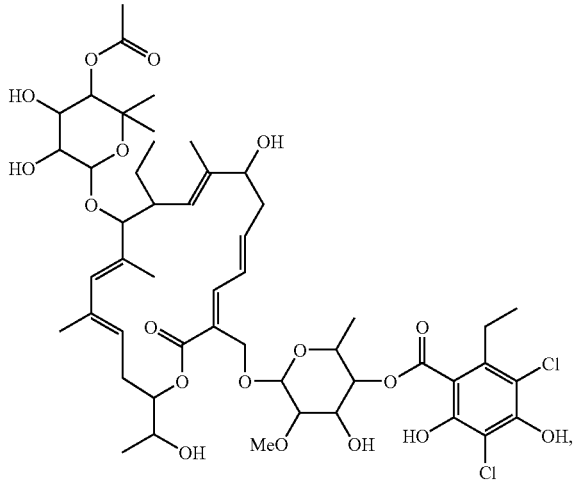
The term "MIC" or "minimum inhibitory concentration" refers to the lowest concentration of an antibiotic that is needed to inhibit growth of a bacterial isolate in vitro. A common method for determining the MIC of an antibiotic is to prepare several tubes containing serial dilutions of the antibiotic, that are then inoculated with the bacterial isolate of interest. The MIC of an antibiotic can be determined from the tube with the lowest concentration that shows no turbidity (no growth).

The term "MIC50" refers to the lowest concentration of antibiotic required to inhibit the growth of 50% of the bacterial strains tested within a given bacterial species.

The term "MIC90" refers to the lowest concentration of antibiotic required to inhibit the growth of 90% of the bacterial strains tested within a given bacterial species.

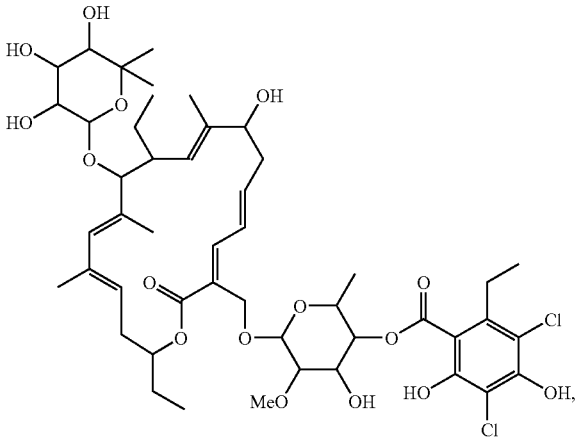
As used herein and unless otherwise indicated, the term "mixture of tiacumicins" refers to a composition containing at least one macrolide compound from the family of compounds known tiacumicins. In another embodiment, the term "mixture of tiacumicins" includes a mixture containing at least one member of the compounds known tiacumicins and a Compound of Formula I, wherein the Compound of Formula I is present in an amount of about 50%, 60%, 70%, 80%, 90%, 95%, 99%, 99.9%, or 99.99% by weight. In particular, the term "mixture of tiacumicins" refers to a compositions comprising a Compound of Formula I, wherein the Compound of Formula I has a relative retention time ("RTT") ratio of 1.0, and further comprising at least one of the following compounds:

Compound 101



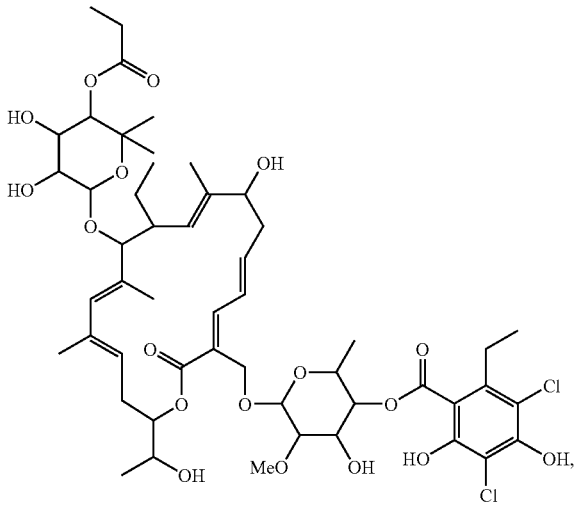
RRT ratio 0.71

Compound 102



RRT ratio 0.81

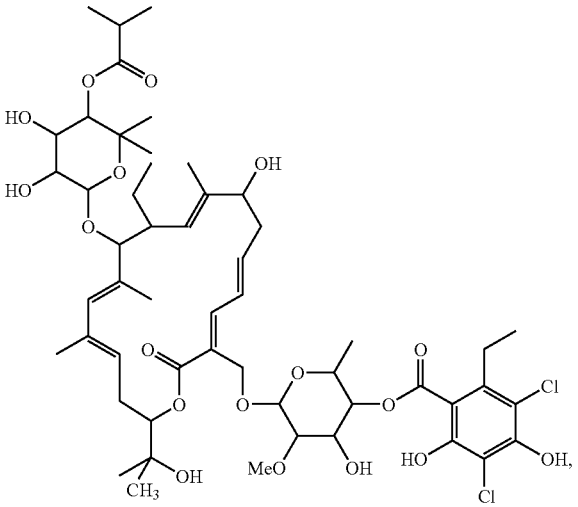
Compound 103



RRT ratio 0.84

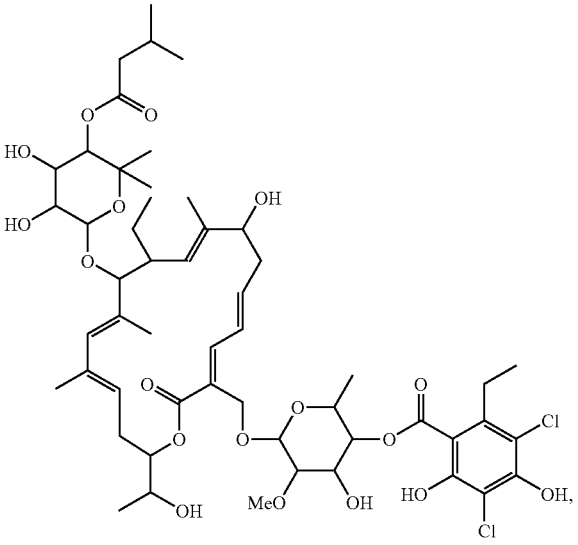
-continued

Compound 104



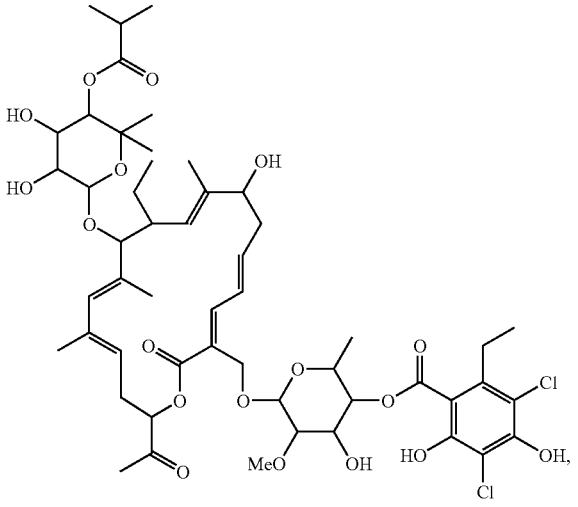
RRT ratio 1.13

Compound 105



RRT ratio 1.19

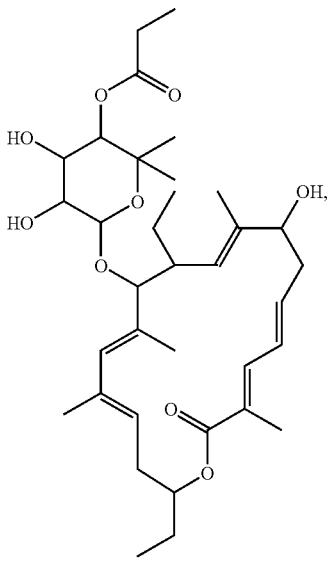
Compound 106



RRT ratio 1.24

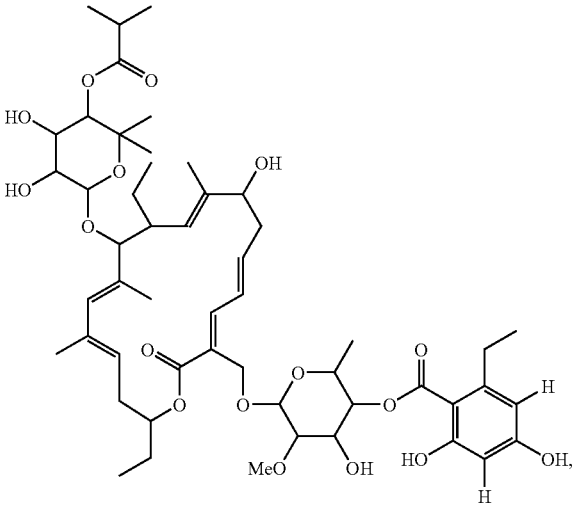
-continued

Compound 107



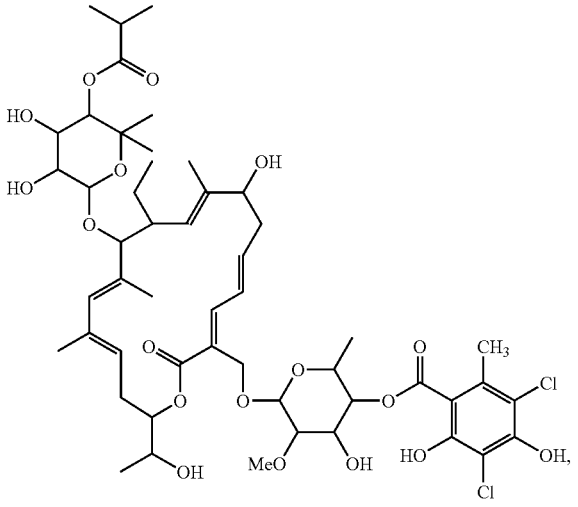
RRT ratio 1.39

Compound 108



RRT ratio 1.48

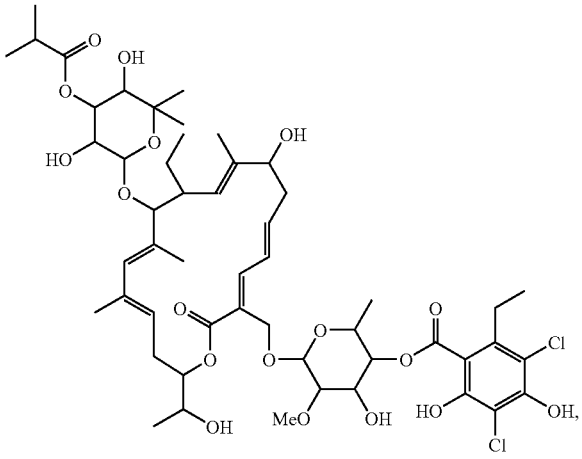
Compound 109



RTT ratio 0.89

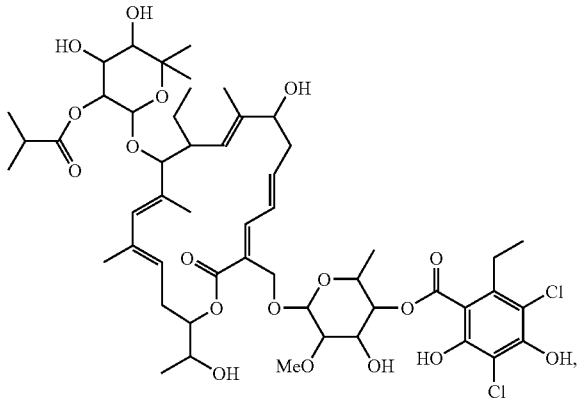
-continued

Compound 110



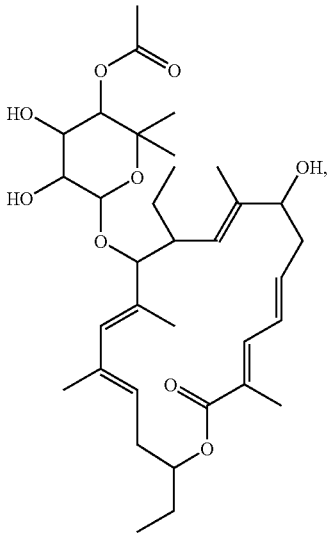
RTT ratio 0.92

Compound 111



RTT ratio 0.95

Compound 112



RTT ratio 1.10

In certain illustrative embodiments, when compound 109 is present in the mixture optionally one of compounds 110, 111, and/or 112 is also present in the mixture. Compound 109 is also sometimes referred to as Lipiarmycin A4. Compound 110 is also sometimes referred to as Tiacumicin F. Compound 111 is also sometimes referred to as Tiacumicin C. Compound 112 is also sometimes referred to as Tiacumicin A.

As used herein, and unless otherwise indicated, the terms "optically pure," "stereomerically pure," and "substantially stereomerically pure" are used interchangeably and mean one stereoisomer of a compound or a composition that comprises one stereoisomer of a compound and is substantially free of other stereoisomer(s) of that compound. For example, a stereomerically pure compound or composition of a compound having one chiral center will be substantially free of the opposite enantiomer of the compound. A stereomerically pure compound or composition of a compound having two chiral centers will be substantially free of other diastereomers of the compound. A typical stereomerically pure compound comprises greater than about 80% by weight of one stereoisomer of the compound and less than about 20% by weight of other stereoisomers of the compound, more preferably greater than about 90% by weight of one stereoisomer of the compound and less than about 10% by weight of the other stereoisomers of the compound, even more preferably greater than about 95% by weight of one stereoisomer of the compound and less than about 5% by weight of the other stereoisomers of the compound, and most preferably greater than about 97% by weight of one stereoisomer of the compound and less than about 3% by weight of the other stereoisomers of the compound.

As used herein and unless otherwise indicated, "pharmaceutically acceptable" refers to materials and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human. Typically, as used herein, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

As used herein and unless otherwise indicated, the term "pharmaceutically acceptable hydrate" means a Compound of the Invention that further includes a stoichiometric or non-stoichiometric amount of water bound by non-covalent intermolecular forces.

As used herein and unless otherwise indicated, the term "pharmaceutically acceptable polymorph" refers to a Compound of the Invention that exists in several distinct forms (e.g., crystalline, amorphous), the invention encompasses all of these forms.

As used herein and unless otherwise indicated, the term "pharmaceutically acceptable prodrug" means a derivative of a modified polymorph of a compound of Formula I that can hydrolyze, oxidize, or otherwise react under biological conditions (in vitro or in vivo) to provide the compound. Examples of prodrugs include, but are not limited to, compounds that comprise biohydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. Other examples of prodrugs include compounds that comprise oligonucleotides, peptides, lipids, aliphatic and aromatic groups, or NO, NO_2, ONO, and ONO_2 moieties. Prodrugs can typically be prepared using well known methods, such as those described in Burger's Medicinal Chemistry and Drug Discovery, 172 178, 949 982 (Manfred E. Wolff ed., 5th ed. 1995), and Design of Prodrugs (H. Bundgaard ed., Elselvier, New York 1985).

The phrase "pharmaceutically acceptable salt(s)," as used herein includes but is not limited to salts of acidic or basic groups that may be present in compounds used in the present compositions. Compounds included in the present compositions that are basic in nature are capable of forming a wide variety of salts with various inorganic and organic acids. The acids that may be used to prepare pharmaceutically acceptable acid addition salts of such basic compounds are those that form non-toxic acid addition salts, i.e., salts containing pharmacologically acceptable anions including, but not limited to, sulfuric, citric, maleic, acetic, oxalic, hydrochloride, hydrobromide, hydroiodide, nitrate, sulfate, bisulfate, phosphate, acid phosphate, isonicotinate, acetate, lactate, salicylate, citrate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucaronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate and pamoate (i.e., 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)) salts. Compounds included in the present compositions that include an amino moiety may form pharmaceutically acceptable salts with various amino acids, in addition to the acids mentioned above. Compounds, included in the present compositions, which are acidic in nature are capable of forming base salts with various pharmacologically acceptable cations. Examples of such salts include alkali metal or alkaline earth metal salts and, particularly, calcium, magnesium, sodium lithium, zinc, potassium, and iron salts.

As used herein and unless otherwise indicated, the term "prophylactically effective" refers to an amount of a Compound or Composition of the Invention or a pharmaceutically acceptable salt, solvate, polymorph, or prodrug thereof causing a reduction of the risk of acquiring a given disease or disorder. Accordingly, the Compounds of the Invention may be used for the prevention of one disease or disorder and concurrently treating another (e.g., prevention of AAC, while treating urinary AAD). In certain embodiments, the compositions of the invention are administered to a patient, preferably a human, as a preventative measure against such diseases. As used herein, "prevention" or "preventing" refers to a reduction of the risk of acquiring a given disease or disorder.

As used herein, the term "subject" can be a mammal, preferably a human or an animal. The subject being treated is a patient in need of treatment.

As used herein and unless otherwise indicated, the phrase "therapeutically effective amount" of a Compound or Composition of the Invention or a pharmaceutically acceptable salt, solvate, polymorph, or prodrug thereof is measured by the therapeutic effectiveness of a compound of the invention, wherein at least one adverse effect of a disorder is ameliorated or alleviated. In one embodiment, the term "therapeutically effective amount" means an amount of a drug or Compound of the Invention that is sufficient to provide the desired local or systemic effect and performance at a reasonable benefit/risk ratio attending any medical treatment. In one embodiment, the phrase "therapeutically effective amount" of a composition of the invention is measured by the therapeutic effectiveness of a compound of the invention to alleviate at least one symptom associated with bacterial or protazoal infections. Surprisingly, the inventors have found that therapeutically effective amounts of the compounds of the invention are useful in treating or preventing bacterial and protazoal infections.

As used herein and unless otherwise indicated, the terms "treatment" or "treating" refer to an amelioration of a disease or disorder, or at least one discernible symptom thereof, preferably associated with a bacterial or protozoal infection. In another embodiment, "treatment" or "treating" refers to an amelioration of at least one measurable physical parameter, not necessarily discernible by the patient. In yet another embodiment, "treatment" or "treating" refers to inhibiting the progression of a disease or disorder, either physically, e.g., stabilization of a discernible symptom, physiologically, for example, stabilization of a physical parameter, or both. In yet another embodiment, "treatment" or "treating" refers to delaying the onset of a disease or disorder.

6.3. Compositions of the Invention for Therapeutic/Prophylactic Administration

The invention encompasses compositions comprising a first polymorph of a Compound of Formula I, a second polymorph of a Compound of Formula I, other polymorphic forms, amorphous form or mixtures thereof of a mixture of tiacumicins with varying amounts of the Compound of Formula I.

The invention further encompasses an antibiotic composition that is a mixture of tiacumicins for use in treating CDAD as well as, AAD and AAC. The mixture of tiacumicins contains about 76 to about 100% of a Compound of Formula I, which belongs to the tiacumicin family of 18-member macrolide.

Due to the activity of the Compounds of the Invention, the compounds are advantageously useful in veterinary and human medicine. The Compounds of the Invention are useful for the treatment or prevention of bacterial and protozoal infections. In some embodiments, the subject has an infection but does not exhibit or manifest any physiological symptoms associated with an infection.

The invention provides methods of treatment and prophylaxis by administration to a patient of a therapeutically effective amount of a composition comprising a crystalline polymorph or amorphous form of a Compound of the Invention. The patient is a mammal, including, but not limited, to an animal such a cow, horse, sheep, pig, chicken, turkey, quail, cat, dog, mouse, rat, rabbit, guinea pig, etc., and is more preferably a human.

The present compositions, which comprise one or more crystalline polymorph or amorphous form of a Compounds of the Invention or a mixture of tiacumicins may be administered by any convenient route, for example, peroral administration, parenteral administration, by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with another biologically active agent. Administration can be systemic or local. Various delivery systems are known, e.g., encapsulation in liposomes, microparticles, microcapsules, capsules, etc., and can be used to administer a compound of the invention. In certain embodiments, more than one Compound of the Invention and mixture of tiacumicins is administered to a patient. Methods of administration include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, oral, sublingual, intranasal, intracerebral, intravaginal, transdermal, rectally, by inhalation, or topically, particularly to the ears, nose, eyes, or skin. The preferred mode of administration is left to the discretion of the practitioner, and will depend in-part upon the site of the medical condition. In most instances, administration will result in the release of the crystalline polymorph or amorphous form of a Compound of the Invention into the bloodstream.

In specific embodiments, it may be desirable to administer one or more crystalline polymorph or amorphous form of a Compound of the Invention locally to the area in need of treatment. This may be achieved, for example, and not by way of limitation, by local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration can be by direct injection at the site (or former site) of an atherosclerotic plaque tissue.

Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent, or via perfusion in a fluorocarbon or synthetic pulmonary surfactant. In certain embodiments, the compounds of the invention can be formulated as a suppository, with traditional binders and vehicles such as triglycerides.

In another embodiment, the a crystalline polymorph or amorphous form of a Compound of the Invention can be delivered in a vesicle, in particular a liposome (see Langer, 1990, Science 249:1527-1533; Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid.).

In yet another embodiment, the compounds of the invention can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, 1987, *CRC Crit. Ref Biomed. Eng.* 14:201; Buchwald et al., 1980, *Surgery* 88:507 Saudek et al., 1989, N. Engl. J. Med. 321:574). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, J. *Macromol. Sci. Rev. Macromol. Chem.* 23:61; see also Levy et al., 1985, *Science* 228:190; During et al., 1989, *Ann. Neurol.* 25:351; Howard et al., 1989, J. *Neurosurg.* 71:105). In yet another embodiment, a controlled-release system can be placed in proximity of the target of the compounds of the invention, e.g., the liver, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, supra, vol. 2, pp. 115-138 (1984)). Other controlled-release systems discussed in the review by Langer, 1990, Science 249:1527-1533) may be used.

The present compositions will contain a therapeutically effective amount of a crystalline polymorph or amorphous form of a Compound of the Invention, optionally more than one crystalline polymorph or amorphous form of a Compound of the Invention, preferably in purified form, together with a suitable amount of a pharmaceutically acceptable vehicle so as to provide the form for proper administration to the patient.

In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "vehicle" refers to a diluent, adjuvant, excipient, or carrier with which a compound of the invention is administered. Such pharmaceutical vehicles can be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The pharmaceutical vehicles can be saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea, and the like. In addition, auxiliary, stabilizing, thickening, lubricating and coloring agents may be used. When administered to a patient, the compounds of the invention and pharmaceutically acceptable vehicles are preferably sterile. Water is a preferred vehicle when the compound of the invention is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid vehicles, particularly for injectable solutions. Suitable pharmaceutical vehicles also include excipients such as starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The present compositions, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

The present compositions can take the form of solutions, suspensions, emulsion, tablets, pills, pellets, capsules, capsules containing liquids, powders, sustained-release formulations, suppositories, emulsions, aerosols, sprays, suspensions, or any other form suitable for use. In one embodiment, the pharmaceutically acceptable vehicle is a capsule (see e.g., U.S. Pat. No. 5,698,155). Other examples of suitable pharmaceutical vehicles are described in "Remington's Pharmaceutical Sciences" by A. R. Gennaro.

In a preferred embodiment, the crystalline polymorph or amorphous form of a Compound of the Invention is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, a crystalline polymorph or amorphous form of a Compound of the Invention for intravenous administration is a solution in sterile isotonic aqueous buffer. Where necessary, the compositions may also include a solubilizing agent. Compositions for intravenous administration may optionally include a local anesthetic such as lidocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the crystalline polymorph or amorphous form of a Compound of the Invention is to be administered by infusion, it can be dispensed, for example, with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the compound of the invention is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

It is preferred that the compositions of the invention be administered orally. Compositions for oral delivery may be in the form of tablets, lozenges, aqueous or oily suspensions, granules, powders, emulsions, capsules, syrups, or elixirs, for example. Orally administered compositions may contain one or more optionally agents, for example, sweetening agents such as fructose, aspartame or saccharin; flavoring agents such as peppermint, oil of wintergreen, or cherry; coloring agents; and preserving agents, to provide a pharmaceutically palatable preparation. Moreover, where in tablet or pill form, the compositions may be coated to delay disintegration and absorption in the gastrointestinal tract thereby providing a sustained action over an extended period of time. Selectively permeable membranes surrounding an osmotically active driving compound are also suitable for orally administered crystalline polymorph or amorphous form of a Compound of the Invention. In these later platforms, fluid from the environment surrounding the capsule

is imbibed by the driving compound, which swells to displace the agent or agent composition through an aperture. These delivery platforms can provide an essentially zero order delivery profile as opposed to the spiked profiles of immediate release formulations. A time delay material such as glycerol monostearate or glycerol stearate may also be used. Oral compositions can include standard vehicles such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Such vehicles are preferably of pharmaceutical grade.

The amount of a crystalline polymorph or amorphous form of a Compound of the Invention that will be effective in the treatment of a particular disorder or condition disclosed herein will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, in vitro or in vivo assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the compositions will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. However, suitable dosage ranges for oral administration are generally about 0.001 milligram to 1000 milligrams of a compound of the invention per kilogram body weight. In specific preferred embodiments of the invention, the oral dose is 0.01 milligram to 500 milligrams per kilogram body weight, more preferably 0.1 milligram to 100 milligrams per kilogram body weight, more preferably 0.5 milligram to 50 milligrams per kilogram body weight, and yet more preferably 1 milligram to 10 milligrams per kilogram body weight. In a most preferred embodiment, the oral dose is 1 milligram of a crystalline polymorph or amorphous form of a Compound of the Invention per kilogram body weight. The dosage amounts described herein refer to total amounts administered; that is, if more than one compound of the invention is administered, the preferred dosages correspond to the total amount of the compounds of the invention administered. Oral compositions preferably contain 10% to 95% active ingredient by weight.

Suitable dosage ranges for intravenous (i.v.) administration are 0.001 milligram to 1000 milligrams per kilogram body weight, 0.1 milligram to 100 milligrams per kilogram body weight, and 1 milligram to 10 milligrams per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Suppositories generally contain 0.01 milligram to 50 milligrams of a compound of the invention per kilogram body weight and comprise active ingredient in the range of 0.5% to 10% by weight. Recommended dosages for intradermal, intramuscular, intraperitoneal, subcutaneous, epidural, sublingual, intracerebral, intravaginal, transdermal administration or administration by inhalation are in the range of 0.001 milligram to 1000 milligrams per kilogram of body weight. Suitable doses of the compounds of the invention for topical administration are in the range of 0.001 milligram to 1 milligram, depending on the area to which the compound is administered. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems. Such animal models and systems are well known in the art.

The invention also provides pharmaceutical packs or kits comprising one or more containers filled with one or more crystalline polymorph or amorphous form of a Compound of the Invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In a certain embodiment, the kit contains more than one crystalline polymorph or amorphous form of a Compound of the Invention.

The crystalline polymorph or amorphous form of a Compound of the Invention is preferably assayed in vitro and in vivo, for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays can be used to determine whether administration of a specific compound of the invention or a combination of compounds of the invention is preferred for lowering fatty acid synthesis. The compounds of the invention may also be demonstrated to be effective and safe using animal model systems.

Other methods will be known to the skilled artisan and are within the scope of the invention.

6.4. General Synthesis of the Compounds of the Invention

The 18-membered macrocycles and analogs thereof are produced by fermentation. Cultivation of *Dactylosporangium aurantiacum* subspecies *hamdenensis* AB 718C-41 NRRL 18085 for the production of the tiacumicins is carried out in a medium containing carbon sources, inorganic salts and other organic ingredients with one or more absorbents under proper aeration conditions and mixing in a sterile environment.

The microorganism to produce the active antibacterial agents was identified as belonging to the family Actinoplanaceae, genus *Dactylosporangium* (*J. Antibiotics,* 1987, 40: 567-574 and U.S. Pat. No. 4,918,174). It has been designated *Dactylasporangium aurantiacum* subspecies *hamdenensis* 718C-41. The subculture was obtained from the ARS Patent Collection of the Northern Regional Research Center, United States Department of Agriculture, 1815 North University Street, Peoria, Ill. 61604, U.S.A., where it was assigned accession number NRRL 18085. The characteristics of strain AB 718C-41 are given in the *Journal of Antibiotics,* 1987, 40: 567-574 and U.S. Pat. No. 4,918,174.

This invention encompasses the composition of novel antibiotic agents, Tiacumicins, by submerged aerobic fermentation of the microorganism *Dactylosporangium aurantiacum* subspecies *hamdenensis*. The production method is disclosed in WO 2004/014295 A2, which is hereby incorporated by reference.

7. EXAMPLES

7.1. Preparation of the Crude Mixtures of Tiacumicins and the Subsequent Crystallization of Certain Polymorphs of the Mixtures

In an illustrative embodiment, a mixture of tiacumicins containing the Compound of Formula I is prepared by a process comprising:

(i) culturing a microorganism in a nutrient medium to accumulate the mixture in the nutrient medium; and

(ii) isolating the mixture from the nutrient medium; wherein the nutrient medium comprises an adsorbent to adsorb the mixture.

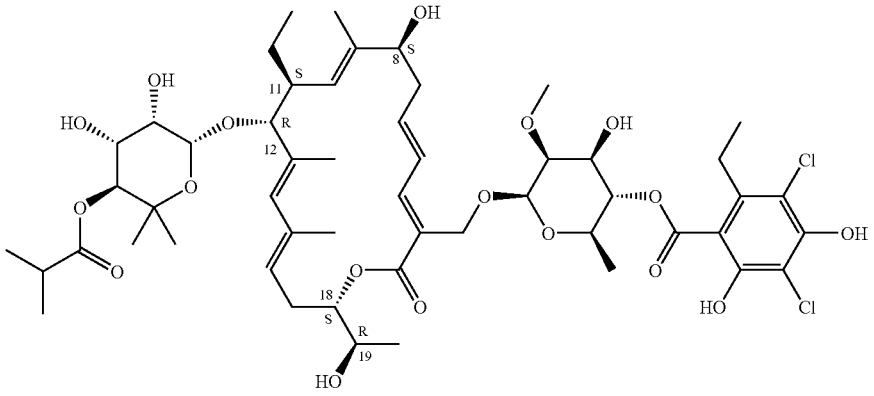
The nutrient medium preferably comprises from about 0.5 to about 15% of the adsorbent by weight. The absorbent is preferably an adsorbent resin. More preferably, the adsorbent resin is Amberlite®, XAD16, XAD16HP, XAD2, XAD7HP, XAD1180, XAD1600, IRC50, or Duolite® XAD761. The microorganism is preferably *Dactylosporangium aurantiacum* subspecies *hamdenensis*. The nutrient medium comprises the following combination based on weight: from about 0.2% to about 10% of glucose, from

about 0.02% to about 0.5% of K_2HPO_4, from about 0.02% to about 0.5% of $MgSO_4.7H_2O$, from about 0.01% to about 0.3% of KCl, from about 0.1% to about 2% of $CaCO_3$, from about 0.05% to about 2% of casamino acid, from about 0.05% to about 2% of yeast extract, and from about 0.5% to about 15% of XAD-16 resin. The culturing step is preferably conducted at a temperature from about 25° C. to about 35° C. and at a pH from about 6.0 to about 8.0.

Upon completion of fermentation, the solid mass (including the adsorbent resin) is separated from the broth by sieving. The solid mass is eluted with organic solvents such as, for example, ethyl acetate then concentrated under reduced pressure.

7.2. Structure of R-Tiacumicin B

The structure of the R-Tiacumicin B (the major most active component) is shown below in Formula I. The X-ray crystal structure of the R-Tiacumicin B was obtained as a colorless, parallelepiped-shaped crystal (0.08×0.14×0.22 mm) grown in aqueous methanol. This x-ray structure confirms the structure shown below. The official chemical name is 3-[[[6-Deoxy-4-O-(3,5-dichloro-2-ethyl-4,6-dihydroxybenzoyl)-2-O-methyl-β-D-mannopyranosyl]oxy]-methyl]-12(R)-[[6-deoxy-5-C-methyl-4-O-(2-methyl-1-oxopropyl)-β-D-lyxo-hexopyranosyl]oxy]-11(S)-ethyl-8(S)-hydroxy-18(S)-(1(R)-hydroxyethyl)-9,13,15-trimethyloxacyclooctadeca-3,5,9,13,15-pentaene-2-one.



7.2.1 Analytical Data of R-Tiacumicin B

The analytical data of R-Tiacumicin B (which is almost entirely (i.e., >90%) R-Tiacumicin).

mp 166-169° C. (white needle from isopropanol);

$[\alpha]_D^{20}$-6.9 (c 2.0, MeOH);

MS m/z (ESI) 1079.7(M+Na)$^+$;

^{1}H NMR (400 MHz, CD_3OD) δ 7.21 (d, 1H), 6.59 (dd, 1H), 5.95 (ddd, 1H), 5.83 (br s, 1H), 5.57 (t, 1H), 5.13 (br d, 1H), 5.09 (t, 1H), 5.02 (d, 1H), 4.71 (m, 1H), 4.71 (br s, 1H), 4.64 (br s, 1H), 4.61 (d, 1H), 4.42 (d, 1H), 4.23 (m, 1H), 4.02 (pentet, 1H), 3.92 (dd, 1H), 3.73 (m, 2H), 3.70 (d, 1H), 3.56 (s, 3H), 3.52-3.56 (m, 2H), 2.92 (m, 2H), 2.64-2.76 (m, 3H), 2.59 (heptet, 1H), 2.49 (ddd, 1H), 2.42 (ddd, 1H), 2.01 (dq, 1H), 1.81 (s, 3H), 1.76 (s, 3H), 1.65 (s, 3H), 1.35 (d, 3H), 1.29 (m, 1H), 1.20 (t, 3H), 1.19 (d, 3H), 1.17 (d, 3H), 1.16 (d, 3 H), 1.14 (s, 3H), 1.12 (s, 3H), 0.87 (t, 3H);

^{13}C NMR (100 MHz, CD_3OD) δ 178.4, 169.7, 169.1, 154.6, 153.9, 146.2, 143.7, 141.9, 137.1, 137.0, 136.4, 134.6, 128.5, 126.9, 125.6, 124.6, 114.8, 112.8, 108.8, 102.3, 97.2, 94.3, 82.5, 78.6, 76.9, 75.9, 74.5, 73.5, 73.2, 72.8, 71.6, 70.5, 68.3, 63.9, 62.2, 42.5, 37.3, 35.4, 28.7, 28.3, 26.9, 26.4, 20.3, 19.6, 19.2, 18.7, 18.2, 17.6, 15.5, 14.6, 14.0, 11.4.

7.3. Preparation of a First Polymorph of R-Tiacumicin B

Another illustrative embodiment of the invention comprises a process for producing a polymorph of a Compound of Formula I from a mixture of tiacumicins comprising the steps of:

a) dissolving a crude mixture of tiacumicins containing from about 76% to about 100% of a Compound of Formula I in a minimum amount of solution comprising methanol, water, acetonitrile, acetic acid, or isopropyl alcohol mixtures thereof;

b) allowing the solution of a) to evaporate while standing at room temperature (e.g., about 22° C.) for 3 to 7 days to precipitate a first polymorph of a Compound of Formula I; and

c) separating the polymorph from the solution by techniques known in the art.

7.3.1. Illustrative Example 1 of the Preparation of a Polymorph of R-Tiacumicin B

After the fermentation process as described for example in Section 7.1, the crude material was purified by reverse phase chromatography using a Biotage Flash 75 L system containing a 1.2 kg, Biotage KP-C18-HS silica column, eluted with 70:30:1, MeOH/H_2O/AcOH. The collected fractions containing 75-80% of Compound of Formula I were combined and concentrated to one-third of the original volume to produce a precipitate. The precipitate is filtered and washed with water. The solid was dried under high vacuum to afford an off-white powder. HPLC analysis showed the powder contains about 78% of Compound of Formula I as a major product and a mixture of tiacumicins as the minor component.

The mixture of tiacumicins containing about 78% of Compound of Formula I (i.e., 50 mg) was dissolved in 2 mL of methanol followed by addition of 1 mL of water. The solution was allowed to evaporate, while standing at room temperature for 7 days to produce a crystalline precipitate. The crystal is separated from the solution by filtration. After methanol/water recrystallization, the crystals contain about 90% of Compound of Formula I based on HPLC.

7.3.2. Illustrative Example 2 of the Preparation of a Polymorph of R-Tiacumicin

After the fermentation process as described for example in Section 7.1, the crude material was purified by reverse phase chromatography using a Biotage Flash 150 system

containing a 3.75 kg, Biotage KP-C18-HS silica column, eluted with 52:48:1, EtOH/H_2O/AcOH. The collected fractions containing about 80-88% of Compound of Formula I were combined and concentrated to one-third the original volume to produce a precipitate. The precipitate was filtered and washed with water. The solid was dried under high vacuum. HPLC analysis showed the powder contains 85.4% of Compound of Formula I as a major product and a mixture of tiacumicins as the minor component.

The mixture containing about 85% of Compound of Formula I (i.e., 1000 mg) was dissolved in 20 mL of a mixture of methanol and water at ratios 1:1 methanol water. The solution was allowed to evaporate/stand at room temperature for 3 days to produce a polymorph crystalline precipitate. The crystal was separated from the solution by filtration.

The composition obtained is a mixture containing a first polymorph of a Compound of Formula I, and at least one of the tiacumicin compounds based on HPLC analysis. The composition has a melting point of 165-169° C.

7.3.3. Illustrative Example 3 of the Preparation of a Polymorph of R-Tiacumicin

After the fermentation process as described for example in Section 7.1, the crude material was purified by reverse phase chromatography using a Biotage Flash 75 L system containing a 1.2 kg, Biotage KP-C18-HS silica column, eluted with MeOH/H_2O/AcOH 67:33:4 to 70:30:1. The collected fractions containing >90% of Compound of Formula I was combined and concentrated to one-third volume. The precipitate was filtered and washed with water. The solid was dried under high vacuum. HPLC analysis showed the powder contains 94.0% of Compound of Formula I.

The solid was tested by X-ray diffraction (XRD) and Differential Scanning Calorimetry (DSC) (See FIGS. **2** and **4**). The X-ray diffraction of the solid shows peaks at angles 2θ of 7.7°, 15.0°, and 18.8°±0.1 indicating the solid is the form of a first polymorph of a Compound of Formula I. The DSC plot shows an endothermic curve starting at about at 169° C. and peak at 177° C.

7.3.4. Illustrative Example 4 of the Preparation of a Polymorph of R-Tiacumicin

After the fermentation process as described for example in Section 7.1, the crude material was purified by reverse phase chromatography using a Biotage Flash 75 L system containing a 1.2 kg, Biotage KP-C18-HS silica column, eluted with 52:48:1, EtOH/H_2O/AcOH. The collected fractions containing >90% of Compound of Formula I were combined, one-third volume of water was added and left at room temperature overnight. The precipitate was filtered and washed with water. The solid was dried under high vacuum. HPLC analysis showed the powder contains 94.7% of Compound of Formula I.

The powder containing 94.7% of Compound of Formula I (i.e., 98 mg) was dissolved in 3 mL of methanol and then 1 mL of water was added. The solution was allowed to evaporate and stand at room temperature for 7 days to produce a crystalline precipitate. The crystals were separated from the solution by filtration and washed with methanol/water 3:1. The crystals were analyzed by X-ray diffraction.

Composition of the precipitate is a mixture comprising a Compound of Formula I based on HPLC analysis with a melting point of 166-169° C.

7.3.5. Illustrative Example 5 of the Preparation of a Polymorph of R-Tiacumicin

After the fermentation process as described for example in Section 7.1, the mixture was purified on a column, and a 0.06 gm of a mixture of tiacumicins was dissolved in 16 mL of methanol and 4 mL of water in a 20 mL vial. The vial is covered with parafilm, and pinholes were punched through. The covered vial is placed in a desiccator and stored at room temperature for ten days. Parafilm cover is then removed, and the vial is returned to desiccator. Crystalline material is produced within three to five days after the parafilm is removed. The crystalline material is washed with a solution of methanol and water and the Compound of Formula I was isolated in 75.6%.

X-ray powder diffraction pattern of the crystalline material is shown in FIG. **3** included 2θ of 7.7°, 15.0°, and 18.0°.

7.3.6. Illustrative Example 6 of the Preparation of a Polymorph of R-Tiacumicin

Preparation of a Polymorph from Isopropanol

After the fermentation process as described for example in Section 7.1, the crude material was purified by reverse phase chromatography using a Biotage Flash 150 system containing a 3.75 kg, Biotage KP-C18-HS silica column, eluted with 52:48:1, EtOH/H_2O/AcOH. The collected fractions containing 80-88% of Compound of Formula I were combined and concentrated to one-third of the original volume to produce a precipitate. The precipitate was filtered and washed with water. The solid was dried under high vacuum. HPLC analysis showed the powder contains 85.4% of Compound of Formula I.

The powder containing 85.4% Compound of Formula I (i.e., 2000 mg) was dissolved in 900 mL of isopropanol. The solution was heated to increase solubility and then filtered to remove insoluble materials. The clear solution was allowed to evaporate/stand at room temperature for 14 days to produce a crystalline precipitate. The crystal is separated from the solution by filtration.

Composition of the precipitate is a mixture comprising Compound of Formula I and at least one of other related substances based on HPLC analysis with mp of 163-165° C.

X-ray diffraction of the precipitate shows peaks at angles 2θ of 7.6° and 15.4°.

7.3.7. Illustrative Example 7 of the Preparation of a Polymorph of R-Tiacumicin

After the fermentation process as described for example in Section 7.1, and column purification, a mixture of Compound of Formula I, >90%, 15 g) was dissolved in minimum amount of methanol (from about 20 mL to about 30 mL), the solution was triturated with isopropanol (~100 mL) to produce a polymorph. The solid is separated from the solution by filtration with melting point of 165-168° C.

The XRD diagram shows a distinct polymorph pattern comprising 2 theta values of 7.5°, 15.2°, 15.7°, 18.6° 18.7°.

7.3.8. Illustrative Example 5 of the Preparation of a Polymorph of R-Tiacumicin

Preparation of a Polymorph from Acetonitrile

The mixture of tiacumicins obtained as described above and (85.44% of Compound of Formula I, 1000 mg) was dissolved in 30 mL of acetonitrile. The solution was allowed to evaporate and stand at room temperature for 12 days to produce a crystalline precipitate. The crystal is separated from the solution by filtration, and exhibits a melting point of 165-169° C.

The XRD diagram of this crystal shows the pattern of a polymorph comprising 2 theta values of 7.8°, 15.1°, 18.8°.

7.4. Preparation of Other Polymorphs of R-Tiacumicin

Another illustrative embodiment of the invention comprises a process for producing a polymorph of a Compound of Formula I comprising the steps of:

a) dissolving crude mixture of tiacumicins containing from about 78 to about 100% of a Compound of Formula I in a minimum amount of ethyl acetate;

b) allowing the solution to evaporate and stand at room temperature for 3 to 7 days to precipitate a polymorph; and

c) separating polymorph from the solution

7.4.1. Illustrative Example 1 of the Preparation of a Polymorph of R-Tiacumicin

Preparation of Polymorph from Ethyl Acetate

After the fermentation process as described for example in Section 7.1, the crude material was purified by reverse phase chromatography using a Biotage Flash 150 system containing a 3.75 kg, Biotage KP-C18-HS silica column, eluted with 52:48:1, EtOH/H_2O/AcOH. The collected fractions containing 70-88% of Compound of Formula I was combined and concentrated to one-third volume to produce a precipitate. The precipitate is filtered and washed with water. The solid was dried under high vacuum. HPLC analysis showed the powder contains 85.4% of Compound of Formula I.

This crude tiacumicin mixture (1000 mg) was then dissolved in 30 mL of ethyl acetate. The solution was allowed to evaporate and stand at room temperature for 12 days to produce a crystalline precipitate of Polymorph B of the Compound of Formula I. The crystals were separated from the solution by filtration. The crystals have a melting point of about 153-156° C., which confirm a different polymorphic form from the first polymorph.

7.4.2. Illustrative Example 2 of the Preparation of a Polymorph of R-Tiacumicin

Preparation of a Polymorph from Methanol and Isopropanol.

After the fermentation process as described for example in Section 7.1, six different batches of crude material of varying amounts of Compound of Formula I were combined such that the combination has an average of 91% of Compound of Formula I. The combination was dissolved in methanol and concentrated by rotary evaporation. The concentrated solution is then mixed with isopropanol, filtered, and dried by vacuum to produce a white powder with a melting point of 156-160° C.

X-ray powder diffraction of the white powder is shown in FIG. **6** comprising 2 theta values of 7.5°, 15.4°, and 18.7°.

7.4.3. Illustrative Example 3 of the Preparation of a Polymorph of R-Tiacumicin

Preparation of Polymorph B From Chloroform

After the fermentation process as described for example in Section 7.1, a crude material of tiacumicins containing Compound of Formula I was dissolved in chloroform and concentrated by evaporation at room temperature to produce a solid with a melting point of 156-160° C.

7.4.4. Illustrative Example 4 of the Preparation of a Polymorph of R-Tiacumicin

Preparation of a Polymorphic Form from Acetone

After the fermentation process as described for example in Section 7.1, a crude material of tiacumicins containing Compound of Formula I was dissolved in acetone and concentrated by evaporation at room temperature to produce a solid with a melting point of 156-160° C.

7.5. Preparation of Amorphous Forms of Compound of Formula I

Preparation of Amorphous Mixture of Tiacumicins

The amorphous mixture of tiacumicins was obtained after column purification without any further processing steps. Alternatively, chloroform or acetone may be added to the mixture of tiacumicins and the solvent is evaporated to form the amorphous product.

X-ray powder diffraction of the product exhibits no defined diffraction peaks.

8. EXPERIMENTAL DATA

8.1. Polymorph Experimental Data

A first polymorph of a Compound of a Compound of Formula I is characterized by Differential Scanning Calorimetry ("DSC") and powder X-Ray Diffraction ("XRD").

The DSC plot of the polymorph shows an endothermic curve at 177° C.

The XRD diagram (reported in FIG. **1**) shows peaks comprising at diffraction angles 2θ of 7.7°, 15.0°, 18.8°. The XRD was analyzed with a Phillips powder Diffractometer by scanning from 20 to 70 degrees two-theta at 1.0 degree per minute using Cu K-alpha radiation, at 35 kV and 20 ma. The instrumental error (variant) is 0.04 (2 theta value).

The melting point of the mixtures containing various amounts of Compound of Formula I is summarized in Table 1. All of the products with at least 85% of a Compound of Formula I in the form of a polymorph appear to have a melting point in the range of 163-169° C. measured by Melting Point apparatus, MEL-TEMP 1001.

TABLE 1

Melting point of polymorph mixtures in different solvent conditions

No.	Compound of Formula I Content (%) of the crystalline material	Mp (° C.)	Crystallization Solvent
1	85	165-169	MeOH/Water
2	85	163-165	Isopropanol
3	85	164-168	Acetonitrile
4	90	165-168	MeOH/Isopropanol
5	94	166-169	MeOH/Water
6	95	166-169	MeOH/Water
7	98	163-164	MeOH/Isopropanol

Composition of the a polymorphic crystal from a mixture comprising Compound of Formula I and optionally at least on compound that is a mixture of tiacumicins based on HPLC analysis with a melting point of 166-169° C.

X-ray diffraction of a polymorphic crystal shows characteristic peaks at angles 2θ of 7.8°, 15.0°, 18.8°, and 23.9°. Table 2 is a listing of the obtained X-ray diffraction peaks for first polymorph of R-Tiacumicin from Experiment 7.2.2.

TABLE 2

X-ray diffraction peaks for a First Polymorph from Experiment 7.3.2.

Two-Theta	Relative Intesity
3.3568	44.0000
3.4400	47.0000
7.7815	112.0000
10.1575	32.0000
13.6023	21.0000
15.0951	139.0000
17.0178	18.0000
18.8458	36.0000
19.3771	9.0000
20.0300	16.0000
20.4842	10.0000
23.9280	136.0000
24.8338	10.0000
25.0889	19.0000
25.7256	10.0000
30.9126	75.0000
31.9970	10.0000
34.4507	30.0000

Table 3 is a listing of the obtained X-ray diffraction peaks for Polymorph from Experiment 7.3.6.

TABLE 3

X-ray diffraction peaks for a Polymorph from Experiment 7.3.6.

Two-Theta	Relative Intensity
3.2978	41.0000
7.5615	400.0000
9.9482	21.0000
15.4289	31.0000
22.0360	20.0000
22.5361	20.0000
24.9507	12.0000
29.5886	10.0000
34.8526	19.0000
37.7092	17.0000
40.4361	13.0000
42.2446	18.0000

8.2 Second Polymorph of R-Tiacumicin Experimental Data

A second polymorph of Compound of Formula I is also characterized by Differential Scanning Calorimetry (DSC) and powder X-Ray Diffraction (XRD).

The DSC plot of polymorph B shows an endothermic curve at 158° C. The XRD diagram (reported in FIG. **5**) shows peaks comprising at the values of the diffraction angles 2-theta of 7.6°, 15.4° and 18.8°. Polymorph B has a melting point in the range of 153-156° C. measured by Melting Point apparatus, MEL-TEMP 1001.

It is believed that crystalline polymorphic forms of Compounds of Formula I other than the above-discussed A and B exist and are disclosed herein. These crystalline polymorphic forms, including A and B, and the amorphous form or mixtures thereof contain varying amounts of Compound of Formula I and in certain cases mixtures of tiacumicins can be advantageously used in the production of medicinal preparations having antibiotic activity.

X-ray powder diffraction of the crystals is shown in FIG. **3** with peaks at angles 2θ of 7.5°, 15.7°, and 18.9°±0.04 indicating the presence of Polymorph B.

The DSC plot of Polymorph B shows an endothermic curve starting at about at 150° C. and peak at 158° C.

Table 4 is a summary of the various data that was isolated for illustrative crystallization lots.

TABLE 4

Data Summarizing Various Lots

No.	Compound of Formula I Content (%)	Mp (° C.)	DSC (° C.) Peak	XRD (2 theta)	Crystallization Solvent
1	76.3	155-158		7.7, 15.0, 18.8,	MeOH/Water
2	85.3	159-164	180	7.8, 14.9, 18.8,	MeOH/Water
3	85.4	163-165		7.6, 15.4	Iso-propanol (IPA)
4	85.4	164-168		7.9, 15.0, 18.8	Acetonitrile
5	85.4	153-156		7.5, 15.7, 18.9	EtOAc
6	90	165-168		7.5, 15.2, 15.7, 18.6	MeOH/ Isopropanol
7	97.2	160-163	177	7.4, 15.4, 18.7	IPA
8	94.0	166-169	177	7.6, 15.1, 18.6	MeOH/Water
9	97.2	167-173	187	7.8, 14.8, 18.8	MeOH/Water
10	96.7		160	7.5, 15.4, 18.8	EtOAc
11	98.3	163-164	178	7.7, 15.0, 18.8	MeOH/IPA

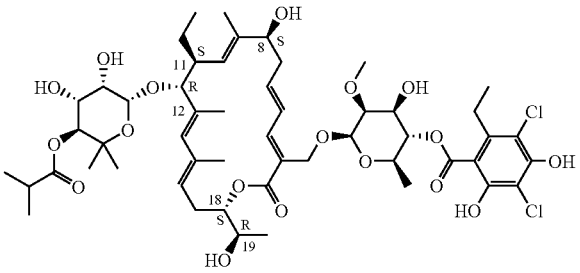
The present invention is not to be limited in scope by the specific embodiments disclosed in the examples which are intended as illustrations of a few aspects of the invention and any embodiments which are functionally equivalent are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art and are intended to fall within the appended claims.

A number of references have been cited, the entire disclosures of which are incorporated herein by reference.

What is claimed is:

1. A polymorphic form of a compound of Formula I:

Formula I



characterized by a powder x-ray diffraction pattern wherein said x-ray diffraction pattern comprises peaks at diffraction angles 2θ of 7.7°, 15.0°, and 18.8°±0.2 as said peaks are set forth in FIG. **1**.

2. A solid dosage form comprising the polymorphic form of a compound of Formula I of claim **1**.

3. The solid dosage form of claim **2**, wherein the polymorphic form of a compound of Formula I is present in at least about 75% to about 99.99% of the total weight.

4. The solid dosage form of claim **2**, wherein the polymorphic form of a compound of Formula I is present in at least about 85% of the total weight.

5. The solid dosage form of claim **2**, wherein the polymorphic form of a compound of Formula I is present in at least about 90% of the total weight.

6. The solid dosage form of claim **2**, wherein the polymorphic form of a compound of Formula I is present in at least about 95% of the total weight.

7. The solid dosage form of claim **2**, wherein the polymorphic form of a compound of Formula I is present in at least about 99% of the total weight.

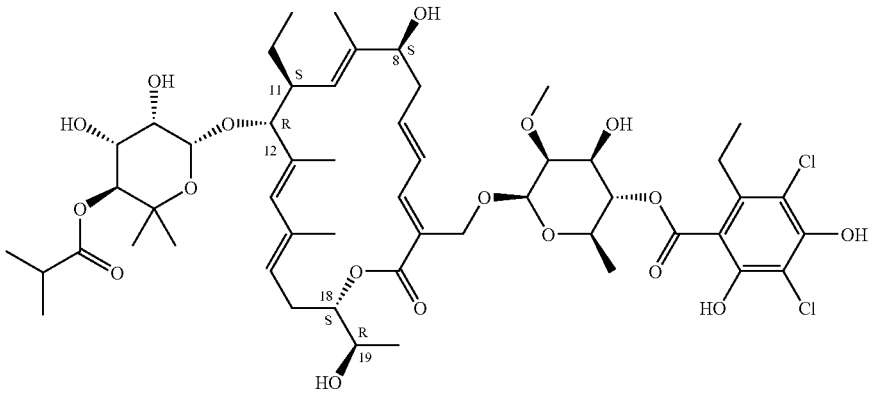
8. The polymorphic form of the compound of Formula I according to claim **1** characterized by a DSC endotherm in the range of about 174° C. to about 186° C.

9. A solid dosage form comprising the polymorphic form of a compound of Formula I of claim **8**.

10. The solid dosage form of claim **9**, wherein the polymorphic form of a compound of Formula I is present from about 75% to about 99.99% of the total weight.

11. A polymorphic form of a compound of Formula I:

Formula I



characterized by:

(i) a powder x-ray diffraction pattern wherein said x-ray diffraction Pattern comprises peaks at diffraction angles 2θ of 7.7°, 15.0°, and 18.8°±0.2 as said peaks are set forth in FIG. **1**; and

(ii) a DSC endotherm in the range of about 174° C. to about 186° C.

12. A solid dosage form comprising the polymorphic form of a compound of Formula I of claim **11**.

13. The solid dosage form of claim **12** wherein the polymorphic form of a compound of Formula I is present from about 75% to about 99.99% of the total weight.

14. The solid dosage form of claim **12**, wherein the polymorphic form of a compound of Formula I is present in about 90% of the total weight.

15. A pharmaceutical composition comprising the solid dosage form of claim **2**.

16. A pharmaceutical composition comprising the solid dosage form of claim **9**.

17. The pharmaceutical composition of claim **15** further comprising a pharmaceutically acceptable excipient.

18. The pharmaceutical composition of claim **16** further comprising a pharmaceutically acceptable excipient.

19. The polymorphic form of the compound of Formula I of claim **1** characterized by a diffraction pattern as set forth in FIG. **1**.

20. The polymorphic form of the compound of Formula I of claim **11** characterized by a diffraction pattern as set forth in FIG. **1**.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,378,508 B2
APPLICATION NO. : 11/831886
DATED : May 27, 2008
INVENTOR(S) : Yu-Hung Chiu et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the title page, please delete Item [63]:

"Continuation-in-part of application No. PCT/US2005/002887, filed on Jan. 31, 2005."

In column 27, line 33 to line 34, delete "FIGS. 2 and 4" and insert --FIG. 2--

In column 29, line 37 to line 38, delete "is shown in FIG. 6 comprising" and insert --comprises--

In column 31, line 26, delete "(reported in FIG. 5)"

Signed and Sealed this

Twenty-second Day of June, 2010

David J. Kappos

David J. Kappos
Director of the United States Patent and Trademark Office

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,378,508 B2
APPLICATION NO. : 11/831886
DATED : May 27, 2008
INVENTOR(S) : Yu-Hung Chiu et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In column 28, line 9, delete "FIG. 3 included 2θ of 7.7°, 15.0°, and 18.0°" and insert --FIG. 1 included 2θ of 7.7°, 15.0°, and 18.8°--

Signed and Sealed this
Eighth Day of February, 2011

David J. Kappos

David J. Kappos
Director of the United States Patent and Trademark Office

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,378,508 B2
APPLICATION NO. : 11/831886
DATED : May 27, 2008
INVENTOR(S) : Yu-Hung Chiu et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Specification:

In column 1, lines 6-7, delete "is a continuation-in-part application of PCT Application PCT/US05/02887, filed Jan. 31, 2005, and"

In column 31, lines 39-41, delete "is shown in FIG. 3 with peaks at angles 2θ of 7.5°, 15.7°, and 18.9° ± 0.04 indicating" and insert -- with peaks at angles 2θ of 7.5°, 15.7°, and 18.9° ± 0.04 indicates --

Signed and Sealed this
Sixth Day of December, 2016

Michelle K. Lee

Michelle K. Lee
Director of the United States Patent and Trademark Office

EXHIBIT 4

US007863249B2

(12) **United States Patent**
Chiu et al.

(10) **Patent No.: US 7,863,249 B2**
(45) **Date of Patent: *Jan. 4, 2011**

(54) **MACROLIDE POLYMORPHS, COMPOSITIONS COMPRISING SUCH POLYMORPHS, AND METHODS OF USE AND MANUFACTURE THEREOF**

(75) Inventors: **Yu-Hung Chiu**, San Diego, CA (US); **Tessie Mary Che**, San Diego, CA (US); **Alex Romero**, San Diego, CA (US); **Yoshi Ichikawa**, San Diego, CA (US); **Youe-Kong Shue**, Carlsbad, CA (US)

(73) Assignee: **Optimer Pharmaceuticals, Inc.**, San Diego, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 391 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **12/101,552**

(22) Filed: **Apr. 11, 2008**

(65) **Prior Publication Data**

US 2008/0194497 A1 Aug. 14, 2008

Related U.S. Application Data

(63) Continuation of application No. 11/831,886, filed on Jul. 31, 2007, now Pat. No. 7,378,508.

(60) Provisional application No. 60/881,950, filed on Jan. 22, 2007.

(51) **Int. Cl.**
A61K 31/7032 (2006.01)
A61K 31/7048 (2006.01)
C07H 17/08 (2006.01)

(52) **U.S. Cl.** **514/28**; 536/7.1

(58) **Field of Classification Search** ..... None
See application file for complete search history.

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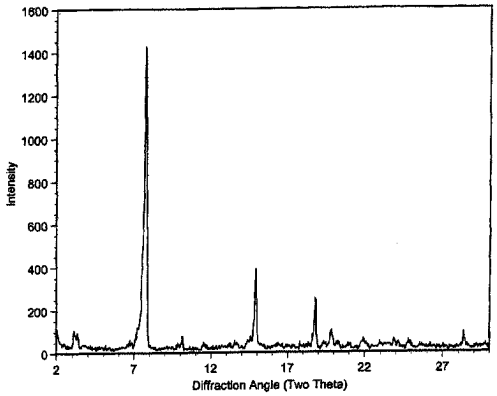
Primary Examiner—Eric S Olson
(74) *Attorney, Agent, or Firm*—Morgan Lewis & Bockius LLP

(57) **ABSTRACT**

The invention relates to novel forms of compounds displaying broad spectrum antibiotic activity, especially crystalline polymorphic forms and amorphous forms of such compounds, compositions comprising such crystalline polymorphic forms and amorphous forms of such compounds, processes for manufacture and use thereof. The compounds and compositions of the invention are useful in the pharmaceutical industry, for example, in the treatment or prevention of diseases or disorders associated with the use of antibiotics, chemotherapies, or antiviral therapies, including, but not limited to, colitis, for example, pseudo-membranous colitis; antibiotic associated diarrhea; and infections due to *Clostridium difficile* ("*C. difficile*"), *Clostridium perfringens* ("*C. perfringens*"), *Staphylococcus* species, for example, methicillin-resistant *Staphylococcus*, or *Enterococcus* including Vancomycin-resistant enterococci.

17 Claims, 3 Drawing Sheets



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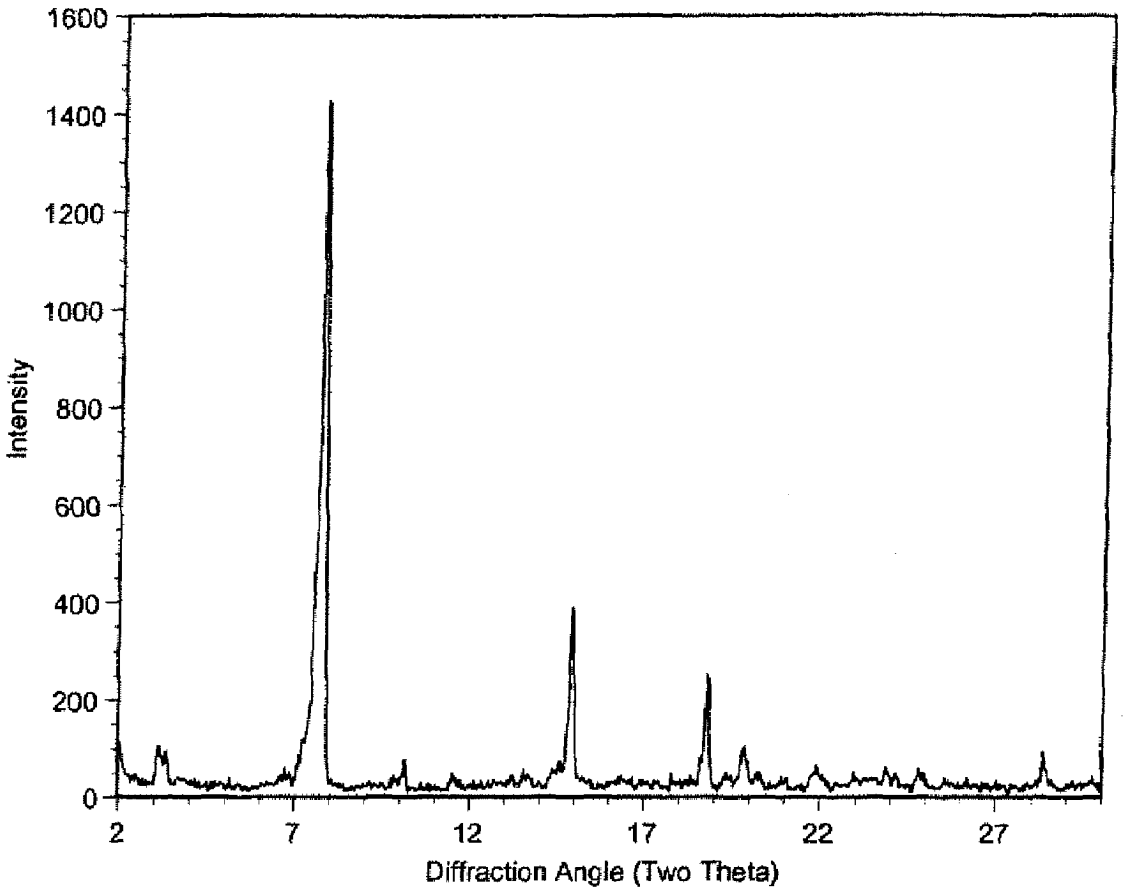
1600
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600
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Intensity
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7
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22
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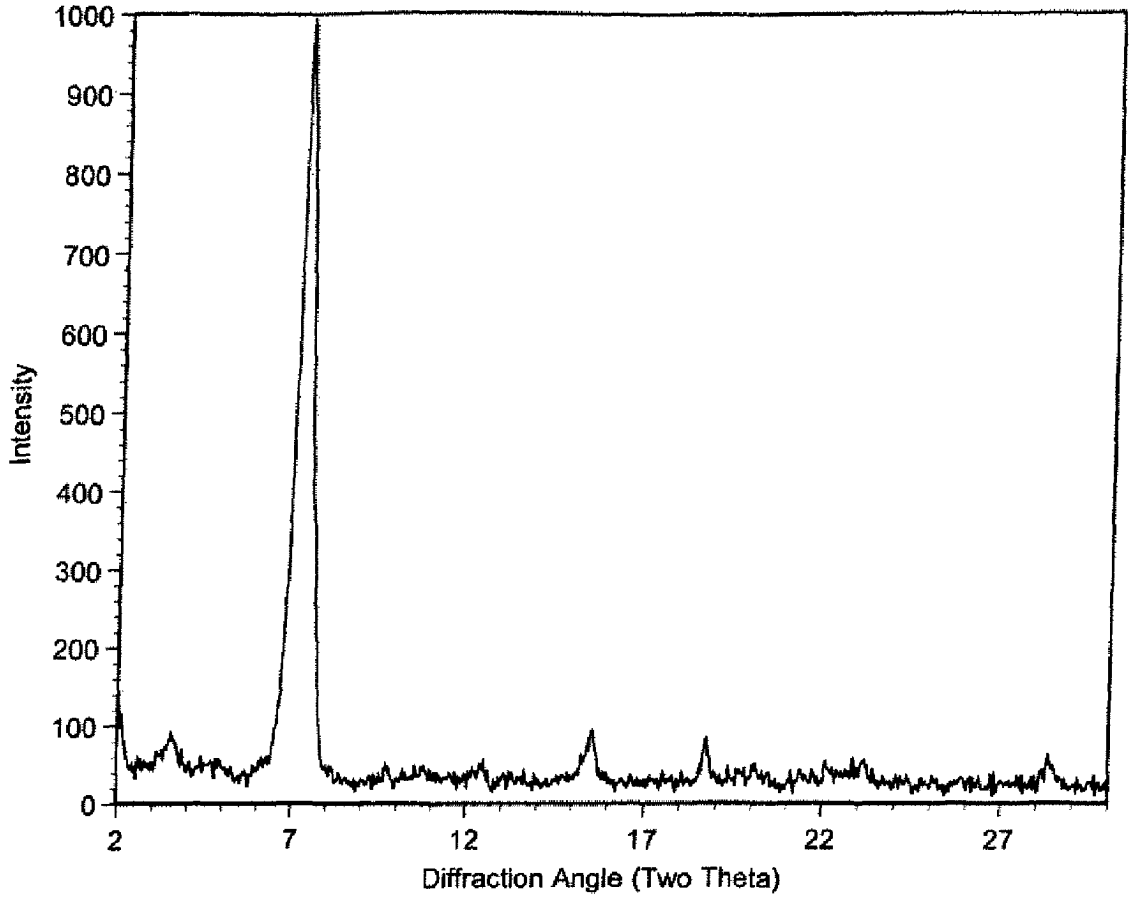
Diffraction Angle (Two Theta)

Figure 1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Intensity
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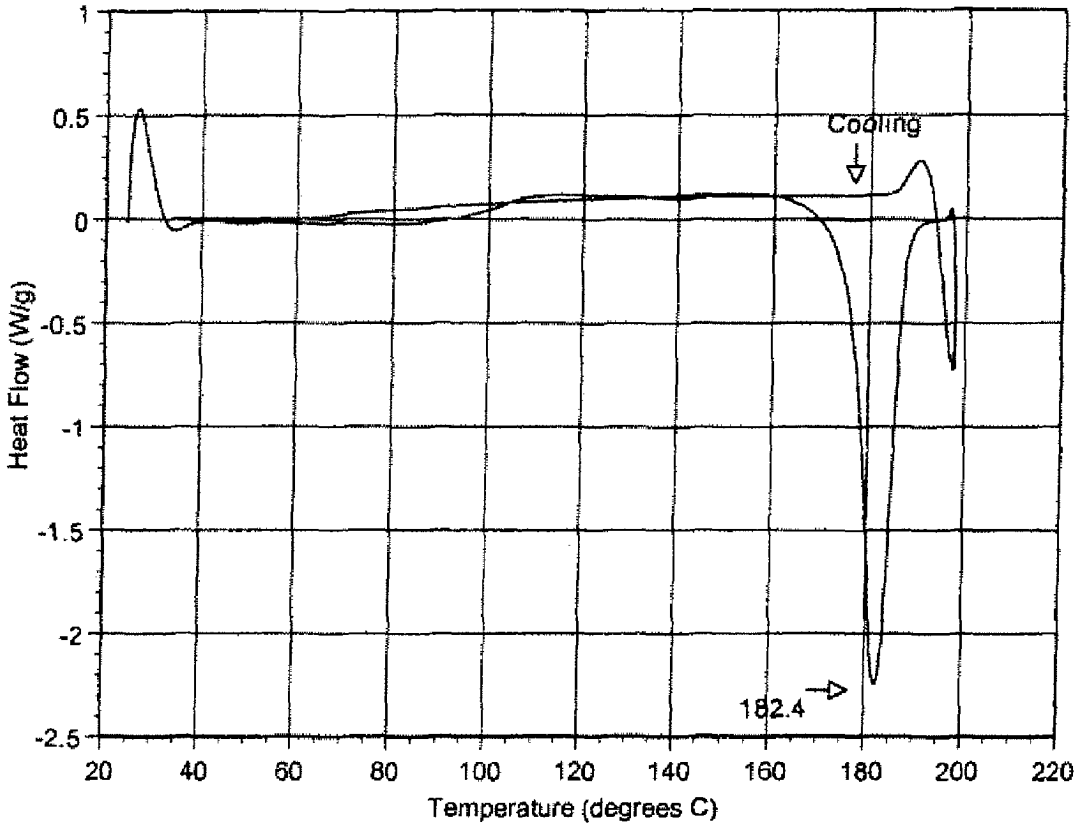
Diffraction Angle (Two Theta)

Figure 2


Heat Flow (W/g)
Temperature (degrees C)
Cooling
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-0.5
-1
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-2
-2.5
20
40
60
80
100
120
140
160
180
200
220

Figure 3

MACROLIDE POLYMORPHS, COMPOSITIONS COMPRISING SUCH POLYMORPHS, AND METHODS OF USE AND MANUFACTURE THEREOF

1. RELATED APPLICATIONS

The present application is a continuation of U.S. patent application Ser. No. 11/831,886, filed Jul. 31, 2007, now U.S. Pat. No. 7,378,508, and claims the benefit of U.S. provisional patent Application No. 60/881,950, filed Jan. 22, 2007, the entire disclosures of each are herein incorporated by reference.

2. FIELD OF THE INVENTION

The invention encompasses novel forms of compounds displaying broad spectrum antibiotic activity, especially crystalline polymorphic forms and amorphous forms of such compounds, compositions comprising such crystalline polymorphic forms and amorphous forms of such compounds, processes for manufacture and use thereof. The compounds and compositions of the invention are useful in the medical and pharmaceutical industry, for example, in the treatment or prevention of diseases or disorders associated with the use of antibiotics, chemotherapies, or antiviral therapies, including, but not limited to, colitis, for example, pseudo-membranous colitis; antibiotic associated diarrhea; and infections due to *Clostridium difficile* ("*C. difficile*"), *Clostridium perfringens* ("*C. perfringens*"), *Staphylococcus* species, for example, methicillin-resistant *Staphylococcus*, or *Enterococcus* including Vancomycin-resistant enterococci.

3. BACKGROUND OF THE INVENTION

Antibiotic-associated diarrhea ("AAD") diseases are caused by toxin producing strains of *C. difficile, Staphylococcus aureus* ("*S. aureus*") including methicillin-resistant *Staphylococcus aureus* ("MRSA") and *C. perfringens*. AAD represents a major economic burden to the healthcare system that is conservatively estimated at $3-6 billion per year in excess hospital costs in the United States alone.

AAD is a significant problem in hospitals and long-term care facilities. *C. difficile* is the leading cause of AAD in the hospital setting, accounting for approximately 20% of cases of AAD and the majority of cases of antibiotic-associated colitis ("AAC"). The rising incidence of *C. difficile* associated diarrhea ("CDAD") has been attributed to the frequent prescribing of broad-spectrum antibiotics to hospitalized patients.

The tiacumicins are a group of 18-membered macrolide antibiotics originally isolated from the fermentation broth of *Dactylosporangium aurantiacum*. The tiacumicins are effective Gram-positive antibiotics. In particular, tiacumicins, specifically Tiacumicin B, show activity against a variety of bacterial pathogens and in particular against *C. difficile*, a Gram-positive bacterium (*Antimicrob. Agents Chemother.*, 1991, 1108-1111). A purification of tiacumicins was carried out in suitable solvents, wherein tiacumicin B exhibited a melting point of 143-145° C. (See, e.g., J. E. Hochlowski, et al., *J. Antibiotics*, vol. XL, no, 5, pages 575-588 (1987)).

The polymorphic behavior of a compound can be of crucial importance in pharmacy and pharmacology. Polymorphs are, by definition, crystals of the same molecule having different physical properties as a result of the order of the molecules in the crystal lattice. The differences in physical properties exhibited by polymorphs affect pharmaceutical parameters such as storage stability, compressibility and density (important in formulation and product manufacturing), and dissolution rates (an important factor in determining bio-availability). Differences in stability can result from changes in chemical reactivity (e.g., differential oxidation, such that a dosage form discolors more rapidly when comprised of one polymorph than when comprised of another polymorph) or mechanical changes (e.g., tablets crumble on storage as a kinetically favored polymorph converts to thermodynamically more stable polymorph) or both (e.g., tablets of one polymorph are more susceptible to breakdown at high humidity). As a result of solubility/dissolution differences, in the extreme case, some polymorphic transitions may result in lack of potency or, at the other extreme, toxicity. In addition, the physical properties of a crystal may be important in processing: for example, one polymorph might be more likely to form solvates or might be difficult to filter and wash free of impurities (i.e., particle shape and size distribution might be different between one polymorph relative to the other).

Each pharmaceutical compound has an optimal therapeutic blood concentration and a lethal concentration. The bio-availability of the compound determines the dosage strength in the drug formulation necessary to obtain the ideal blood level. If the drug can crystallize as two or more polymorphs differing in bio-availability, the optimal dose will depend on the polymorph present in the formulation. Some drugs show a narrow margin between therapeutic and lethal concentrations. Thus, it becomes important for both medical and commercial reasons to produce and market the drug in its most thermodynamically stable polymorph, substantially free of other kinetically favored or disfavored polymorphs.

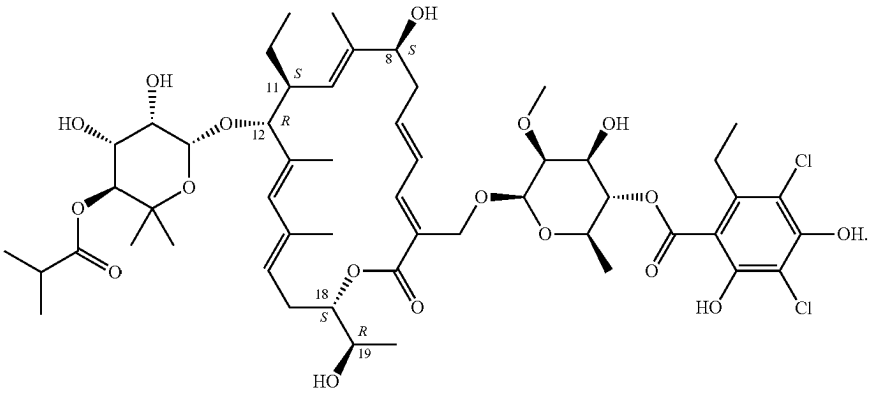
Thus, there is a clear need to develop safe and effective polymorphs of drugs that are efficacious at treating or preventing disorders associated with bacterial pathogens. The present inventors have identified novel crystalline and amorphous forms of 18-membered macrolide compounds that exhibit broad spectrum antibiotic activity.

4. SUMMARY OF THE INVENTION

The invention encompasses novel crystalline and amorphous forms of the macrolide compounds that are useful in treating or preventing bacterial infections and protozoal infections. In an illustrative embodiment, the novel crystalline and amorphous forms of the macrolide compounds of the invention exhibit broad spectrum antibiotic activity. Thus, surprisingly novel crystalline and amorphous forms of the macrolide compounds have been identified, which act as antibiotics possessing a broad spectrum of activity in treating or preventing bacterial infections and protozoal infections, especially those associated with Gram-positive and Gram-negative bacteria and in particular, Gram-positive bacteria.

In one embodiment, the invention encompasses novel crystalline and amorphous forms of the macrolide of Formula I:

Formula I



In another embodiment, the invention encompasses a mixture of compounds with varying amounts of the Compound of Formula I, which forms have the requisite stability for use in preparing pharmaceutical compositions.

In another embodiment, the invention encompasses a polymorph obtained from a mixture of tiacumicins and a Compound of Formula I.

In still another embodiment, the invention encompasses novel crystalline and amorphous forms of the Compound of Formula I.

In another embodiment, the invention encompasses a pharmaceutical composition comprising a Compound of Formula I.

In another embodiment, the invention encompasses a pharmaceutical composition comprising a Compound of Formula I, wherein the Compound of Formula I is present in an amount greater than 90% by weight.

In another embodiment, the invention encompasses a pharmaceutical composition rising one or more novel crystalline and amorphous forms of a Compound of Formula I.

In another embodiment, the invention encompasses a pharmaceutical composition comprising a mixture of tiacumicins and Compound of Formula I.

In another embodiment, the invention encompasses a pharmaceutical composition comprising a mixture of tiacumicins and at least about 75% or more by weight of Compound of Formula I. In another embodiment, the invention encompasses a pharmaceutical composition comprising a mixture of tiacumicins and at least about 80% or more by weight of Compound of Formula I. In another embodiment, the invention encompasses a pharmaceutical composition comprising a mixture of tiacumicins and at least about 85% or more by weight of Compound of Formula I. In another embodiment, the invention encompasses a pharmaceutical composition comprising a mixture of tiacumicins and at least about 90% or more by weight of Compound of Formula I. In another embodiment, the invention encompasses a pharmaceutical composition comprising a mixture of tiacumicins and at least about 95% or more by weight of Compound of Formula I. In another embodiment, the invention encompasses a pharmaceutical composition comprising a mixture of tiacumicins and at least about 99% or more by weight of Compound of Formula I.

The invention also encompasses methods for treating or preventing a disease or disorder including, but not limited to, bacterial infections and protozoal infections comprising administering to a subject, preferably a mammal, in need thereof a therapeutically or prophylactically effective amount of a composition or formulation comprising a compound of the invention.

In one illustrative embodiment, the composition or formulation comprises a mixture of compounds with varying amounts of the Compound of Formula I. In another embodiment, the composition or formulation comprises a mixture of tiacumicins and a Compound of Formula I. In still another embodiment, the composition or formulation comprises novel crystalline and amorphous forms of the Compound of Formula I. In still another embodiment, the composition or formulation comprises novel crystalline and amorphous forms of the Compound of Formula I and a mixture of tiacumicins.

In another particular embodiment, the disease or disorder to be treated or prevented are caused by toxin producing strains of *C. difficile, Staphylococcus aureus* ("*S. aureus*") including methicillin-resistant *Staphylococcus aureus* ("MRSA") and *C. perfringens*. In another particular embodiment, the disease or disorder to be treated or prevented is antibiotic-associated diarrhea.

5. BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** shows the X-ray powder diffraction patterns of a first polymorph Compound of Formula I produced from methanol and water.

FIG. **2** shows the X-ray powder diffraction patterns of a second polymorph Compound of Formula I produced from ethyl acetate.

FIG. **3** shows the effect of temperature on a mixture of tiacumicins produced from methanol and water. The DSC indicates an endothermic curve beginning at 169° C., and weight loss beginning at 223° C. The endothermic curve at about 177° C. corresponds to the melting of a first polymorph of a Compound of Formula I.

6. DETAILED DESCRIPTION OF THE DRAWINGS

6.1. General Description

The invention broadly encompasses mixtures of compounds with varying amounts of the Compound of Formula I. The inventors have surprisingly determined that the formation of crystalline polymorphic forms and amorphous forms of a Compound of Formula I and optionally mixtures of tiacumicin depends on the selection of the crystallization solvent and on the method and conditions of crystallization or precipitation.

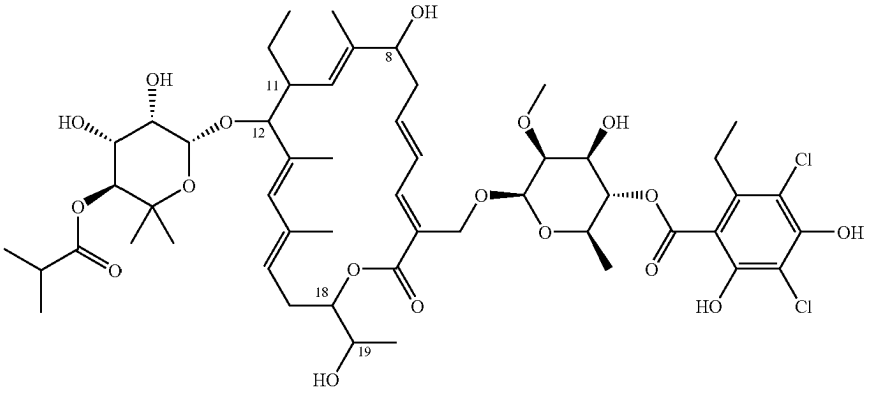
In one embodiment the invention encompasses a mixture of tiacumicins and a Compound of Formula I. In another

embodiment, the invention encompasses novel crystalline and amorphous forms of the Compound of Formula I and optionally a mixture of tiacumicins. In still another embodiment, the invention encompasses novel crystalline and amorphous forms of the Compound of Formula I and a mixture of tiacumicins. In another embodiment, the invention encompasses a mixture of comprising a first polymorph of a Compound of Formula I, a second polymorph of a Compound of Formula I, and other polymorphic forms, amorphous forms and mixtures thereof.

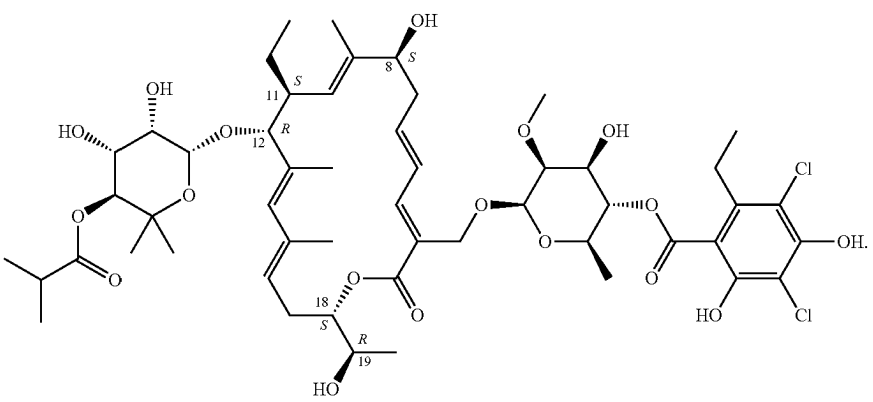
In another particular embodiment, the crystalline polymorphs and amorphous forms are obtained from a mixture of tiacumicins.

In another embodiment, a crystalline polymorph of a Compound of Formula I exhibits a representative powder diffraction pattern comprising at least peaks at the following diffraction angles 2θ of 7.7°, 15.0°, and 18.8°±0.04, preferably ±0.1, more preferably ±0.15, even more preferably ±0.2. In another embodiment, a crystalline polymorph of a Compound of Formula I exhibits a representative powder diffraction pattern comprising at least peaks at the following diffraction angles 2θ of 7.8°, 15.1°, and 18.8°±0.04, preferably ±0.1, more preferably ±0.15, even more preferably ±0.2.

In another embodiment, the polymorph has the chemical structure:



In another embodiment, the polymorph has the chemical structure of a Compound of Formula I:



Formula I

In another embodiment, the polymorph further comprises at least one compound selected from a mixture of tiacumicins.

In another embodiment, the polymorph of Formula I is present in an amount from at least about 75% to about 99.99%.

In another embodiment, the polymorph of Formula I is present in an amount of at least about 75%.

In another embodiment, the polymorph of Formula I is present in an amount of at least about 80%.

In another embodiment, the polymorph of Formula I is present in an amount of at least about 85%.

In another embodiment, the polymorph of Formula I is present in an amount of at least about 90%.

In another embodiment, the polymorph of Formula I is present in an amount of at least about 93%.

In another embodiment, the polymorph of Formula I is present in an amount of at least about 95%.

In another embodiment, the polymorph of Formula is present in an amount of at least about 99%.

In another embodiment, the crystalline polymorph is obtained from a mixture of tiacumicins that exhibits a melting point of about 163° C. to about 169° C. In another embodiment, the crystalline polymorph is obtained from a mixture of tiacumicins that exhibits a melting point of about 160° C. to about 170° C. In another embodiment, the crystalline polymorph is obtained from a mixture of tiacumicins that exhibits a melting point of about 155° C. to about 175° C.

In another embodiment, the crystalline polymorph is obtained from a mixture of tiacumicins and exhibits a DSC endotherm in the range of about 174° C. to about 186° C.; preferably 175-185° C.

In another embodiment, the crystalline polymorph is obtained from a mixture of tiacumicins that exhibits a powder diffraction pattern comprising at least peaks at the following diffraction angles 2θ of 7.7°, 15.0°, and 18.8°±0.04, preferably ±0.1, more preferably ±0.15, even more preferably ±0.2 and exhibits a melting point of about 163° C. to about 169° C.

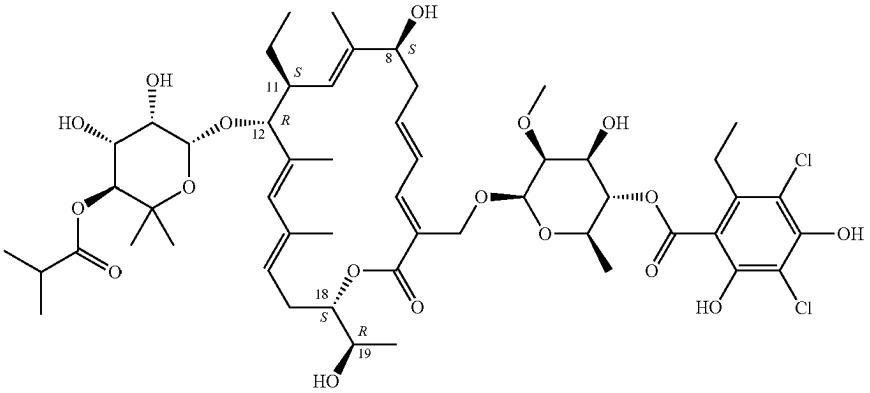
In another embodiment, the crystalline polymorph is obtained from a mixture of tiacumicins that exhibits a powder diffraction pattern comprising at least peaks at the following diffraction angles 2θ of 7.7°, 15.0°, and 18.8°±0.04, preferably ±0.1, more preferably ±0.15, even more preferably ±0.2 and exhibits a melting point of about 160° C. to about 170° C.

Another embodiment encompasses a crystalline polymorph obtained from a mixture of tiacumicins that exhibits a powder diffraction pattern comprising at least peaks at the following diffraction angles 2θ of 7.7°, 15.0°, and 18.8°±0.04, preferably ±0.1, more preferably ±0.15, even more preferably ±0.2. In a particular embodiment, the polymorph has the chemical structure of a Compound of Formula I. In another embodiment, the crystalline polymorph further comprises at least one compound selected from a mixture of tiacumicins.

In another embodiment, a crystalline polymorph is obtained from a mixture of tiacumicins that exhibits a melting point of about 150° C. to about 156° C.

In another embodiment, a crystalline polymorph is obtained from a mixture of tiacumicins that exhibits a powder diffraction pattern comprising at least peaks at the following diffraction angles 2θ of 7.4°, 15.5°, and 18.8°±0.2 and exhibits a melting point of about 150° C. to about 156° C.

Another embodiment of the invention encompasses pharmaceutical compositions comprising a therapeutically or prophylactically effective amount of a crystalline polymorph of a Compound of Formula.



and a pharmaceutically acceptable carrier.

In a particular embodiment, the pharmaceutical composition comprises a first polymorph of a Compound of Formula I, a second polymorph of a Compound of Formula I, other polymorphic forms of a Compound of Formula I, amorphous forms of a Compound of Formula I, and mixtures thereof.

In another embodiment, the crystalline polymorph of the pharmaceutical composition has peaks at the following diffraction angles 2θ of 7.7°, 15.0°, and 18.8°±0.04, preferably ±0.1, more preferably ±0.15, even more preferably ±0.2.

In another embodiment, the crystalline polymorph of the pharmaceutical composition further comprises at least one compound selected from a mixture of tiacumicins.

In another embodiment, the Compound of Formula I is present from at least about 75% to about 99.99%, preferably about 75%, about 85%, about 95%, or about 99%.

In another embodiment, the crystalline polymorph of the pharmaceutical composition exhibits a melting point of about 163° C. to about 169° C.

Another embodiment encompasses a pharmaceutical composition comprising a crystalline polymorph of tiacumicin comprising peaks at the following diffraction angles 2θ of 7.6°, 15.4°, and 18.8°±0.04, preferably ±0.1, more preferably ±0.15, even more preferably ±0.2. In a particular embodiment, the pharmaceutical composition further comprises at least one compound selected from a mixture of tiacumicins. In another particular embodiment, the Compound of Formula I is present from about 75% to about 99.99%, preferably 75%, 85%, 95%, or 99%.

In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 15% of a mixture of tiacumicins. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 10% of a mixture of tiacumicins. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 7% of a mixture of tiacumicins. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 5% of a mixture of tiacumicins. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 1% of a mixture of tiacumicins. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 15% of a mixture of S-Tiacumicin. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 10% of a mixture of S-Tiacumicin. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 7% of a mixture of S-Tiacumicin. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Ti-

acumicin and less than 5% of a mixture of S-Tiacumicin. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 1% of a mixture of S-Tiacumicin. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 15% of a mixture of Lipiarmycin A4. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 10% of a mixture of Lipiarmycin A4. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 7% of a mixture of Lipiarmycin A4. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 5% of a mixture of Lipiarmycin A4. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 1% of a mixture of Lipiarmycin A4.

In another embodiment, the crystalline polymorph of the pharmaceutical composition exhibits a melting point of about 153° C. to about 156° C.

In another embodiment, the therapeutically or prophylactically effective amount is from about 0.01 mg/kg to about 1000 mg/kg, preferably 0.01, 0.1, 1, 2.5, 5, 10, 20, 50, 100, 250, or 500 mg/kg.

In another embodiment, the crystalline polymorph of the pharmaceutical composition is suitable for parenteral administration, preferably intravenous, intramuscular, or intraarterial.

In another embodiment, the crystalline polymorph of the pharmaceutical composition is suitable for peroral administration.

Another embodiment of the invention encompasses a method for treating a bacterial infection comprising administering a pharmaceutical composition comprising a polymorph of the invention to a subject in need thereof.

In a particular embodiment, the bacterial infection is in the gastrointestinal tract, particularly AAC or AAD.

6.2. Definitions

The term "antibiotic-associated condition" refers to a condition resulting when antibiotic therapy disturbs the balance of the microbial flora of the gut, allowing pathogenic organisms such as enterotoxin producing strains of *C. difficile, S. aureus* and *C. perfringens* to flourish. These organisms can cause diarrhea, pseudomembranous colitis, and colitis and are manifested by diarrhea, urgency, abdominal cramps, tenesmus, and fever among other symptoms. Diarrhea, when severe, causes dehydration and the medical complications associated with dehydration.

The term "asymmetrically substituted" refers to a molecular structure in which an atom having four tetrahedral valences is attached to four different atoms or groups. The commonest cases involve the carbon atom. In such cases, two optical isomers (D- and L-enantiomers or R- and S-enantiomers) per carbon atom result which are nonsuperposable mirror images of each other. Many compounds have more than one asymmetric carbon. This results in the possibility of many optical isomers, the number being determined by the formula 2n, where n is the number of asymmetric carbons.

The term "broth" as used herein refers to the fluid culture medium as obtained during or after fermentation. Broth comprises a mixture of water, the desired antibiotic(s), unused nutrients, living or dead organisms, metabolic products, and the adsorbent with or without adsorbed product.

As used herein and unless otherwise indicated, the terms "bacterial infection(s)" and "protozoal infection(s)" are used interchangeably and include bacterial infections and protozoal infections that occur in mammals, fish and birds as well as disorders related to bacterial infections and protozoal infections that may be treated or prevented by antibiotics such as the Compounds of the Invention. Such bacterial infections and protozoal infections, and disorders related to such infections, include the following: disorders associated with the use of antibiotics, chemotherapies, or antiviral therapies, including, but not limited to, colitis, for example, pseudo-membranous colitis, antibiotic associated diarrhea, and infections due to *Clostridium difficile, Clostridium perfringens, Staphylococcus* species, methicillin-resistant *Staphylococcus*, or *Enterococcus* including Vancomycin-resistant *enterococci*; antibiotic-associated diarrhea including those caused by toxin producing strains of *C. difficile, S. aureus* including methicillin-resistant *Staphylococcus aureus*, and *C. perfringens*; and antibiotic-associated colitis; pneumonia, otitis media, sinusitis, bronchitis, tonsillitis and mastoiditis related to infection by *Staphylococcus pneumoniae, Hlaemophilus influenzae, Moraxella catarrhalis, Staphlococcus aureus*, or *Peptostreptococcus* spp.; pharynigis, rheumatic fever and glomerulonephritis related to infection by *Streptococcus pyogenes*, Groups C and G streptococci, *Clostridium diptheriae*, or *Actinobacillus haemolyticum*; respiratory tract infections related to infection by *Mycoplasma pneumoniae, Legionella pneumophila, Streptococcus pneumoniae, Haemophilus influenzae*, or *Chilamydia pneumoniae*; uncomplicated skin and soft tissue infections, abscesses and osteomyelitis, and puerperal fever related to infection by *Staphlococcus aureus*, coagulase-positive staphlococci (e.g., *S. epidermis* and *S. hemolyticus*), *Staphylococcus pyogenes, Streptococcus agalactiae*, Streptococcal groups C-F (minute-colony streptococci), viridans streptococci, *Corynetacterium minutissimum, Clostridium* spp., or *Bartonella henselae*; uncomplicated acute urinary tract infections related to infection by *Staphylococcus saprophyticus* or *Enterococcus* spp.; urethritis and cervicitis; and sexually transmitted diseases related to infection by *Chlamydia trachomatis, Haemophilus ducreyi, Treponema pallidum, Ureaplasma urealyticum*, or *Neiserria gonorrhea*; toxin diseases related to infection by *S. aureus* (food poisoning and Toxic Shock Syndrome), or Groups A, B and C streptococci; ulcers related to infection by *Helicobacter pylori*, systemic febrile syndromes related to infection by *Borrelia recurrentis*; Lyme disease related to infection by *Borrelia burgdorferi*, conjunctivitis, keratitis, and dacrocystitis related to infection by *Chlamydia trachomatis, Neisseria gonorrhoeae, S. aureus, S. pneumoniae, S. pyogenes, H. influenzae*, or *Listeria* spp.; disseminated *Mycobacterium avium* complex (MAC) disease related to infection by *Mycobacterium avium*, or *Mycobacterium intracellulare*; gastroenteritis related to infection by *Campylobacter jejuni*, intestinal protozoa related to infection by *Cryptosporidium* spp.; odontogenic infection related to infection by viridans streptococci; persistent cough related to infection by *Bordetella pertussis*; gas gangrene related to infection by *Clostridium perfringens* or *Bacteroides* spp.; and atherosclerosis related to infection by *Helicobacter pylori* or *Chlamydia pneumoniae*. Bacterial infections and protozoal infections and disorders related to such infections that may be treated or prevented in animals include the following: bovine respiratory disease related to infection by *P. haem., P. multocida, Mycoplasma bovis*, or *Bordetella* spp.; cow enteric disease related to infection by *E. coli* or protozoa (e.g., *coccidia,*

cryptosporidia, etc.); dairy cow mastitis related to infection by *Staph. aureus, Strep. uberis, Strep. agalactiae, Strep. dysgalactiae, Klebsiella* spp. *Corynebacterium*, or *Enterococcus* spp.; swine respiratory disease related to infection by *A. pleuro., P. multocida* or *Mycoplasma* spp.; swine enteric disease related to infection by *E. coli Lawsonia intracellularis, Salmonella*, or *Serpulina hyodyisinteriae*; cow footrot related to infection by *Fusobacterium* spp.; cow metritis related to infection by *E. coli*; cow hairy warts related to infection by *Fusobacterium necrophorum* or *Bacteroides nodosus*; cow pink-eye related to infection by *Moraxela bovis*; cow premature abortion related to infection by protozoa (e.g., neosporium) urinary tract infection in dogs and cats related to infection by *E. coli*; skin and soft tissue infections in dogs and cats related to infection by *Staph. epidermidis, Staph. intermedius*, coagulase neg. *Staph.* or *P. multocida*; and dental or mouth infections in dogs and cats related to infection by *Alcaligenes* spp., *Bacteroides* spp., *Clostridium* spp., *Enterobacter* spp., *Eubacterium, Peptostreptococcus, Porphyromonas*, or *Prevotella*. Other bacterial infections and protozoal infections and disorders related to such infections that may be treated or prevented in accord with the methods of the invention are referred to in Sanford, J. P., et al., "The Sanford Guide To Antimicrobial Therapy," 27th Edition (Antimicrobial Therapy, Inc., 1996).

As used herein and unless otherwise indicated, the term "binders" refers to agents used to impart cohesive qualities to the powdered material. Binders, or "granulators" as they are sometimes known, impart cohesiveness to the tablet formulation, which insures the tablet remaining intact after compression, as well as improving the free-flowing qualities by the formulation of granules of desired hardness and size. Materials commonly used as binders include starch; gelatin; sugars, such as sucrose, glucose, dextrose, molasses, and lactose; natural and synthetic gums, such as acacia, sodium alginate, extract of Irish moss, panwar gum, ghatti gum, mucilage of isapol husks, carboxymethylcellulose, methylcellulose, polyvinylpyrrolidone, Veegum, microcrystalline cellulose, microcrystalline dextrose, amylose, and larch arabogalactan, and the like.

As used herein and unless otherwise indicated, the terms "biohydrolyzable amide," "biohydrolyzable ester," "biohydrolyzable carbamate," "biohydrolyzable carbonate," "biohydrolyzable ureide," "biohydrolyzable phosphate" mean an amide, ester, carbamate, carbonate, ureide, or phosphate, respectively, of a compound that either; 1) does not interfere with the biological activity of the compound but can confer upon that compound advantageous properties in vivo, such as uptake, duration of action, or onset of action; or 2) is biologically inactive but is converted in vivo to the biologically active compound. Examples of biohydrolyzable esters include, but are not limited to, lower alkyl esters, lower acyloxyalkyl esters (such as acetoxylmethyl, acetoxyethyl, aminocarbonyloxy-methyl, pivaloyloxymethyl, and pivaloyloxyethyl esters), lactonyl esters (such as phthalidyl and thiophthalidyl esters), lower alkoxyacyloxyalkyl esters (such as methoxycarbonyloxy-methyl, ethoxycarbonyloxyethyl and isopropoxycarbonyloxyethyl esters), alkoxyalkyl esters, choline esters, and acylamino alkyl esters (such as acetamidomethyl esters). Examples of biohydrolyzable amides include, but are not limited to, lower alkyl amides, a amino acid amides, alkoxyacyl amides, and alkylaminoalkyl-carbonyl amides. Examples of biohydrolyzable carbamates include, but are not limited to, lower alkylamines, substituted ethylenediamines, aminoacids, hydroxyalkylamines, heterocyclic and heteroaromatic amines, and polyether amines.

As used herein and unless otherwise indicated, the term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which a composition is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like.

As used herein and unless otherwise indicated, the term "Compounds of the Invention" means, collectively, a Compound of Formula I and/or pharmaceutically acceptable salts and polymorphs thereof. The compounds of the invention are identified herein by their chemical structure and/or chemical name. Where a compound is referred to by both a chemical structure and a chemical name, and that chemical structure and chemical name conflict, the chemical structure is determinative of the compound's identity. The compounds of the invention may contain one or more chiral centers and/or double bonds and, therefore, exist as stereoisomers, such as double-bond isomers (i.e., geometric isomers), enantiomers, or diastereomers. According to the invention, the chemical structures depicted herein, and therefore the compounds of the invention, encompass all of the corresponding compound's enantiomers and stereoisomers, that is, both the stereomerically pure form (e.g., geometrically pure, enantiomerically pure, or diastereomerically pure) and enantiomeric and stereoisomeric mixtures. Enantiomeric and stereoisomeric mixtures can be resolved into their component enantiomers or stereoisomers by well known methods, such as chiral-phase gas chromatography, chiral-phase high performance liquid chromatography, crystallizing the compound as a chiral salt complex, or crystallizing the compound in a chiral solvent. Enantiomers and stereoisomers can also be obtained from stereomerically- or enantiomerically-pure intermediates, reagents, and catalysts by well known asymmetric synthetic methods. The Compounds of the Invention are preferably substantially stereomerically pure. In a particular embodiment, the term "Compounds of the Invention" refers to a Compound of Formula that is greater than 75% pure, preferably greater than 85% pure, more preferably greater than 95% pure and most preferably greater than 99% pure and polymorphic form (e.g., a polymorph of Compound of Formula I) and amorphous forms thereof.

As used herein and unless otherwise indicated, "diluents" are inert substances added to increase the bulk of the formulation to make the tablet a practical size for compression. Commonly used diluents include calcium phosphate, calcium sulfate, lactose, kaolin, mannitol, sodium chloride, dry starch, powdered sugar, silica, and the like.

As used herein and unless otherwise indicated, "disintegrators" or "disintegrants" are substances that facilitate the breakup or disintegration of tablets after administration. Materials serving as disintegrants have been chemically classified as starches, clays, celluloses, algins, or gums. Other disintegrators include Veegum HV, methylcellulose, agar, bentonite, cellulose and wood products, natural sponge, cation-exchange resins, alginic acid, guar gum, citrus pulp, cross-linked polyvinylpyrrolidone, carboxymethylcellulose, and the like.

When administered to a subject (e.g., to an animal for veterinary use or to a human for clinical use) the compounds of the invention are administered in isolated form. As used herein and unless otherwise indicated, "isolated" means that the compounds of the invention are separated from other components of either (a) a natural source, such as a plant or cell, preferably bacterial culture, or (b) a synthetic organic chemical reaction mixture, preferably, via conventional techniques, the compounds of the invention are purified. As used

herein, "purified" means that when isolated, the isolate contains at least about 70% preferably at least about 80%, more preferably at least about 90%, even more preferably at least about 95%, and most preferably at least about 99% of a compound of the invention by weight of the isolate.

The term "macrolide" or "macrocycle" refers to organic molecules with large ring structures usually containing over 10 atoms.

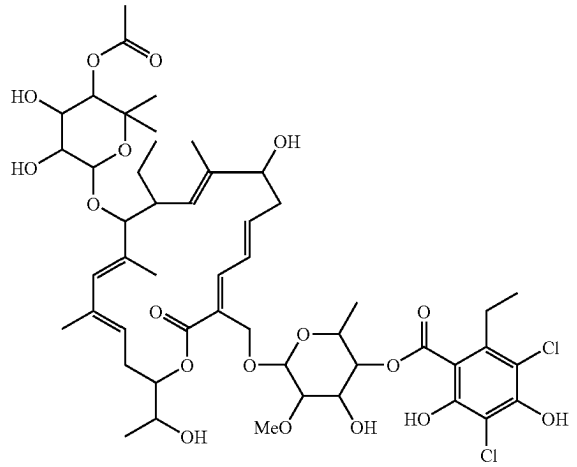
The term "18-membered macrocycles" refers to organic molecules with ring structures containing 18 atoms.

The term "MIC" or "minimum inhibitory concentration" refers to the lowest concentration of an antibiotic that is needed to inhibit growth of a bacterial isolate in vitro. A common method for determining the MIC of an antibiotic is to prepare several tubes containing serial dilutions of the antibiotic, that are then inoculated with the bacterial isolate of interest. The MIC of an antibiotic can be determined from the tube with the lowest concentration that shows no turbidity (no growth).

The term "MIC50" refers to the lowest concentration of antibiotic required to inhibit the growth of 50% of the bacterial strains tested within a given bacterial species.

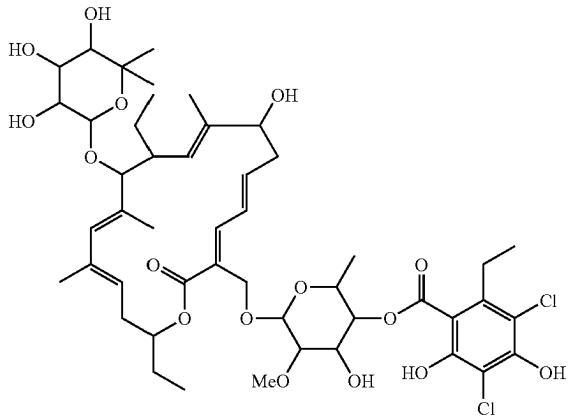
The term "MIC90" refers to the lowest concentration of antibiotic required to inhibit the growth of 90% of the bacterial strains tested within a given bacterial species.

As used herein and unless otherwise indicated, the term "mixture of tiacumicins" refers to a composition containing at least one macrolide compound from the family of compounds known tiacumicins. In another embodiment, the term "mixture of tiacumicins" includes a mixture containing at least one member of the compounds known tiacumicins and a Compound of Formula I, wherein the Compound of Formula I is present in an amount of about 50%, 60%, 70%, 80%, 90%, 95%, 99%, 99.9%, or 99.99% by weight. In particular, the term "mixture of tiacumicins" refers to a compositions comprising a Compound of Formula I, wherein the Compound of Formula I has a relative retention time ("RTT") ratio of 1.0, and farther comprising at least one of the following compounds:

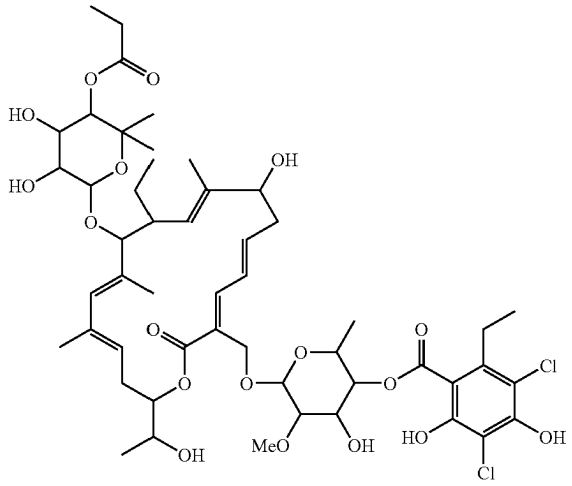


Compound 101, RRT ratio 0.71

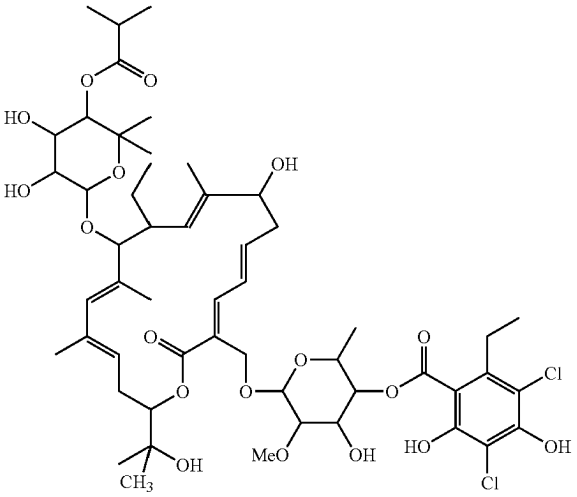
-continued



Compound 102, RRT ratio 0.81

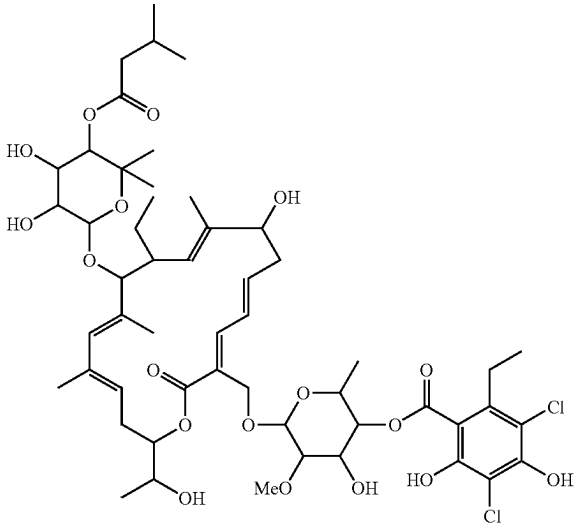


Compound 103, RRT ratio 0.84

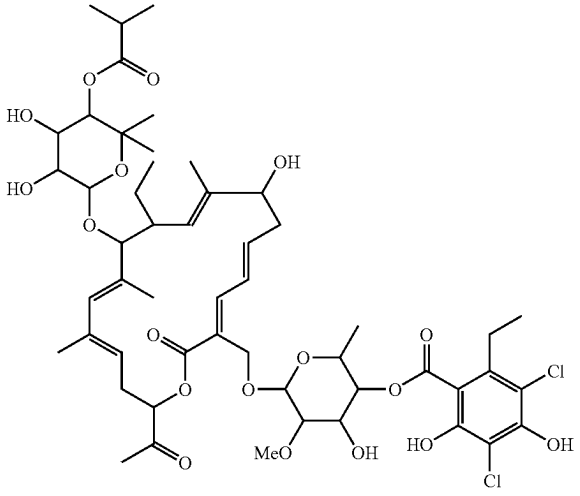


Compound 104, RRT ratio 1.13

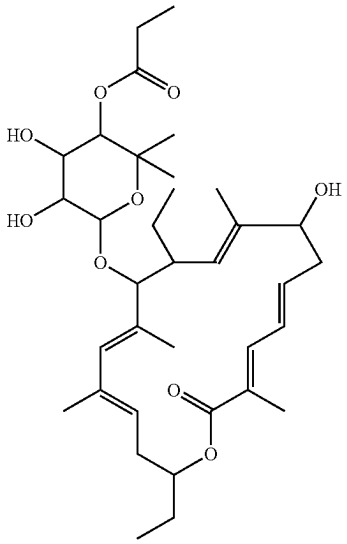
-continued



Compound 105, RRT ratio 1.19

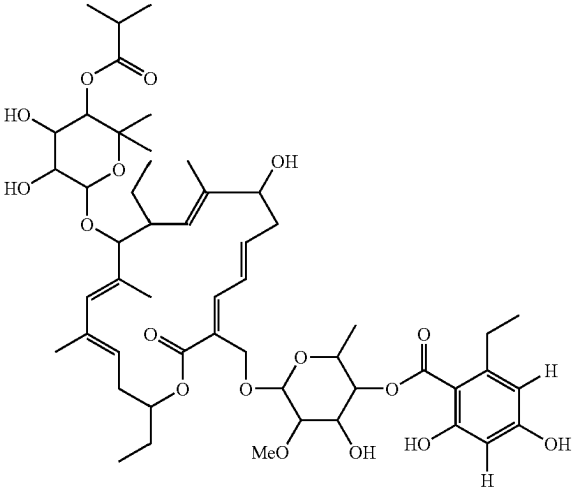


Compound 106, RRT ratio 1.24

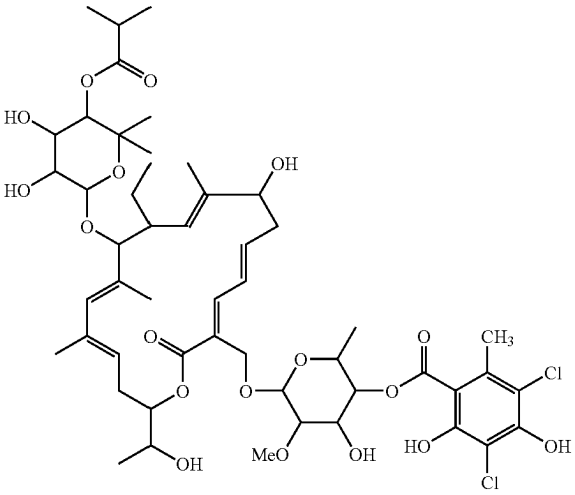


Compound 107, RRT ratio 1.39

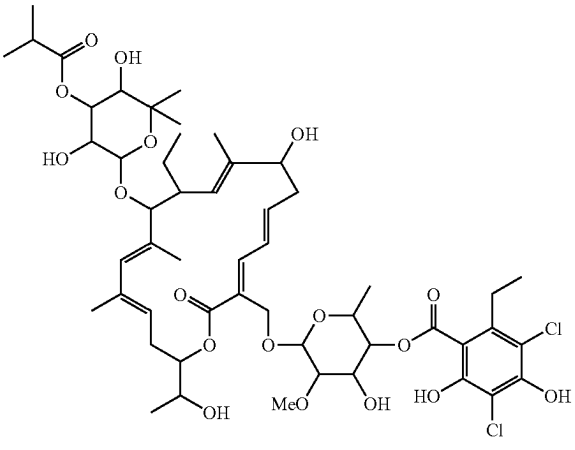
-continued



Compound 108, RRT ratio 1.48

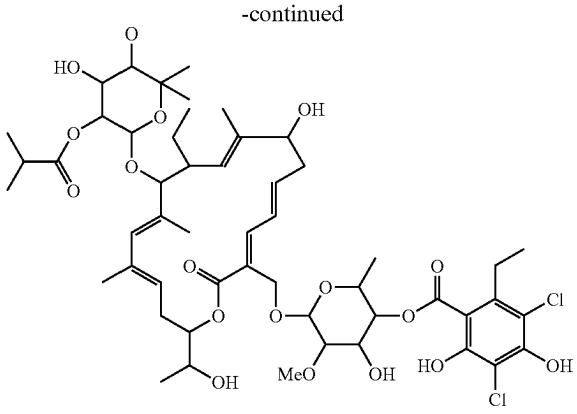


Compound 109, RTT ratio 0.89

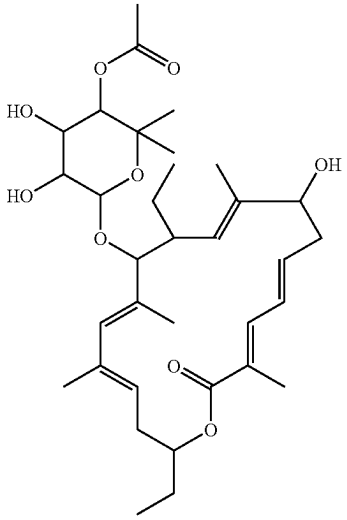


Compound 110, RTT ratio 0.92

-continued



Compound 111, RTT ratio 0.95



Compound 112, RTT ratio 1.10

In certain illustrative embodiments, when compound 109 is present in the mixture optionally one of compounds 110, 111, and/or 112 is also present in the mixture. Compound 109 is also sometimes referred to as Lipiarmycin A4. Compound 110 is also sometimes referred to as Tiacumicin F. Compound III is also sometimes referred to as Tiacumicin C. Compound 112 is also sometimes referred to as Tiacumicin A.

As used herein, and unless otherwise indicated, the terms "optically pure," "stereomerically pure," and "substantially stereomerically pure" are used interchangeably and mean one stereoisomer of a compound or a composition that comprises one stereoisomer of a compound and is substantially free of other stereoisomer(s) of that compound. For example, a stereomerically pure compound or composition of a compound having one chiral center will be substantially free of the opposite enantiomer of the compound. A stereomerically pure compound or composition of a compound having two chiral centers will be substantially free of other diastereomers of the compound. A typical stereomerically pure compound comprises greater than about 80% by weight of one stereoisomer of the compound and less than about 20% by weight of other stereoisomers of the compound, more preferably greater than about 90% by weight of one stereoisomer of the compound and less than about 10% by weight of the other stereoisomers of the compound, even more preferably greater than about 95% by weight of one stereoisomer of the compound and less than about 5% by weight of the other stereoisomers of the compound, and most preferably greater than about 97% by weight of one stereoisomer of the compound and less than about 3% by weight of the other stereoisomers of the compound.

As used herein and unless otherwise indicated, "pharmaceutically acceptable" refers to materials and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human. Typically, as used herein, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

As used herein and unless otherwise indicated, the term "pharmaceutically acceptable hydrate" means a Compound of the Invention that further includes a stoichiometric or non-stoichiometric amount of water bound by non-covalent intermolecular forces.

As used herein and unless otherwise indicated, the term "pharmaceutically acceptable polymorph" refers to a Compound of the Invention that exists in several distinct forms (e.g., crystalline, amorphous), the invention encompasses all of these forms.

As used herein and unless otherwise indicated, the term "pharmaceutically acceptable prodrug" means a derivative of a modified polymorph of a compound of Formula I that can hydrolyze, oxidize, or otherwise react under biological conditions (in vitro or in vivo) to provide the compound. Examples of prodrugs include, but are not limited to, compounds that comprise biohydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. Other examples of prodrugs include compounds that comprise oligonucleotides, peptides, lipids, aliphatic and aromatic groups, or NO, NO_2, ONO, and ONO_2 moieties. Prodrugs can typically be prepared using well known methods, such as those described in Burger's Medicinal Chemistry and Drug Discovery, 172 178, 949 982 (Manfred E. Wolff ed., 5th ed. 1995), and Design of Prodrugs (H. Bundgaard ed., Elselvier, New York 1985).

The phrase "pharmaceutically acceptable salt(s)," as used herein includes but is not limited to salts of acidic or basic groups that may be present in compounds used in the present compositions. Compounds included in the present compositions that are basic in nature are capable of forming a wide variety of salts with various inorganic and organic acids. The acids that may be used to prepare pharmaceutically acceptable acid addition salts of such basic compounds are those that form non-toxic acid addition salts, i.e., salts containing pharmacologically acceptable anions including, but not limited to, sulfuric, citric, maleic, acetic, oxalic, hydrochloride, hydrobromide, hydroiodide, nitrate, sulfate, bisulfate, phosphate, acid phosphate, isonicotinate, acetate, lactate, salicylate, citrate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucaronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate and pamoate (i.e., 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)) salts. Compounds included in the present compositions that include an amino moiety may form pharmaceutically acceptable salts with various amino acids, in addition to the acids mentioned above. Compounds, included in the present compositions, which are acidic in nature are capable of forming base salts with various pharmacologically acceptable cations.

Examples of such salts include alkali metal or alkaline earth metal salts and, particularly, calcium, magnesium, sodium lithium, zinc, potassium, and iron salts.

As used herein and unless otherwise indicated, the term "prophylactically effective" refers to an amount of a Compound or Composition of the Invention or a pharmaceutically acceptable salt, solvate, polymorph, or prodrug thereof causing a reduction of the risk of acquiring a given disease or disorder. Accordingly, the Compounds of the Invention may be used for the prevention of one disease or disorder and concurrently treating another (e.g., prevention of AAC, while treating urinary AAD). In certain embodiments, the compositions of the invention are administered to a patient, preferably a human, as a preventative measure against such diseases. As used herein, "prevention" or "preventing" refers to a reduction of the risk of acquiring a given disease or disorder.

As used herein, the term "subject" can be a mammal, preferably a human or an animal. The subject being treated is a patient in need of treatment.

As used herein and unless otherwise indicated, the phrase "therapeutically effective amount" of a Compound or Composition of the Invention or a pharmaceutically acceptable salt, solvate, polymorph, or prodrug thereof is measured by the therapeutic effectiveness of a compound of the invention, wherein at least one adverse effect of a disorder is ameliorated or alleviated. In one embodiment, the term "therapeutically effective amount" means an amount of a drug or Compound of the Invention that is sufficient to provide the desired local or systemic effect and performance at a reasonable benefit/risk ratio attending any medical treatment. In one embodiment, the phrase "therapeutically effective amount" of a composition of the invention is measured by the therapeutic effectiveness of a compound of the invention to alleviate at least one symptom associated with bacterial or protazoal infections. Surprisingly, the inventors have found that therapeutically effective amounts of the compounds of the invention are useful in treating or preventing bacterial and protazoal infections.

As used herein and unless otherwise indicated, the terms "treatment" or "treating" refer to an amelioration of a disease or disorder, or at least one discernible symptom thereof, preferably associated with a bacterial or protozoal infection. In another embodiment, "treatment" or "treating" refers to an amelioration of at least one measurable physical parameter, not necessarily discernible by the patient. In yet another embodiment, "treatment" or "treating" refers to inhibiting the progression of a disease or disorder, either physically, e.g., stabilization of a discernible symptom, physiologically, for example, stabilization of a physical parameter, or both. In yet another embodiment, "treatment" or "treating" refers to delaying the onset of a disease or disorder.

6.3. Compositions of the Invention for Therapeutic/Prophylactic Administration

The invention encompasses compositions comprising a first polymorph of a Compound of Formula I, a second polymorph of a Compound of Formula I, other polymorphic forms, amorphous form or mixtures thereof of a mixture of tiacumicins with varying amounts of the Compound of Formula I.

The invention further encompasses an antibiotic composition that is a mixture of tiacumicins for use in treating CDAD as well as, AAD and AAC. The mixture of tiacumicins contains about 76 to about 100% of a Compound of Formula I, which belongs to the tiacumicin family of 18-member macrolide.

Due to the activity of the Compounds of the Invention, the compounds are advantageously useful in veterinary and human medicine. The Compounds of the Invention are useful for the treatment or prevention of bacterial and protozoal infections. In some embodiments, the subject has an infection but does not exhibit or manifest any physiological symptoms associated with an infection.

The invention provides methods of treatment and prophylaxis by administration to a patient of a therapeutically effective amount of a composition comprising a crystalline polymorph or amorphous form of a Compound of the Invention. The patient is a mammal, including, but not limited, to an animal such a cow, horse, sheep, pig, chicken, turkey, quail, cat, dog, mouse, rat, rabbit, guinea pig, etc., and is more preferably a human.

The present compositions, which comprise one or more crystalline polymorph or amorphous form of a Compounds of the Invention or a mixture of tiacumicins may be administered by any convenient route, for example, peroral administration, parenteral administration, by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with another biologically active agent. Administration can be systemic or local. Various delivery systems are known, e.g., encapsulation in liposomes, microparticles, microcapsules, capsules, etc., and can be used to administer a compound of the invention. In certain embodiments, more than one Compound of the Invention and mixture of tiacumicins is administered to a patient. Methods of administration include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, oral, sublingual, intranasal, intracerebral, intravaginal, transdermal, rectally, by inhalation, or topically, particularly to the ears, nose, eyes, or skin. The preferred mode of administration is left to the discretion of the practitioner, and will depend in-part upon the site of the medical condition. In most instances, administration will result in the release of the crystalline polymorph or amorphous form of a Compound of the Invention into the bloodstream.

In specific embodiments, it may be desirable to administer one or more crystalline polymorph or amorphous form of a Compound of the Invention locally to the area in need of treatment. This may be achieved, for example, and not by way of limitation, by local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration can be by direct injection at the site (or former site) of an atherosclerotic plaque tissue.

Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent, or via perfusion in a fluorocarbon or synthetic pulmonary surfactant. In certain embodiments, the compounds of the invention can be formulated as a suppository, with traditional binders and vehicles such as triglycerides.

In another embodiment, the a crystalline polymorph or amorphous form of a Compound of the Invention can be delivered in a vesicle, in particular a liposome (see Langer, 1990, Science 249:1527-1533; Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid.).

In yet another embodiment, the compounds of the invention can be delivered in a controlled release system. In one

embodiment, a pump may be used (see Langer, supra; Sefton, 1987, *CRC Crit. Ref. Biomed. Eng.* 14:201; Buchwald et al., 1980, *Surgery* 88:507 Saudek et al., 1989, N. Engl. J. Med. 321:574). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, J. *Macromol. Sci. Rev. Macromol. Chem.* 23:61; see also Levy et al., 1985, *Science* 228:190; During et al., 1989, *Ann. Neurol.* 25:351; Howard et al., 1989, J. *Neurosurg.* 71:105). In yet another embodiment, a controlled-release system can be placed in proximity of the target of the compounds of the invention, e.g., the liver, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, supra, vol. 2, pp. 115-138 (1984)). Other controlled-release systems discussed in the review by Langer, 1990, Science 249:1527-1533) may be used.

The present compositions will contain a therapeutically effective amount of a crystalline polymorph or amorphous form of a Compound of the Invention, optionally more than one crystalline polymorph or amorphous form of a Compound of the Invention, preferably in purified form, together with a suitable amount of a pharmaceutically acceptable vehicle so as to provide the form for proper administration to the patient.

In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "vehicle" refers to a diluent, adjuvant, excipient, or carrier with which a compound of the invention is administered. Such pharmaceutical vehicles can be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The pharmaceutical vehicles can be saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea, and the like. In addition, auxiliary, stabilizing, thickening, lubricating and coloring agents may be used. When administered to a patient, the compounds of the invention and pharmaceutically acceptable vehicles are preferably sterile. Water is a preferred vehicle when the compound of the invention is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid vehicles, particularly for injectable solutions. Suitable pharmaceutical vehicles also include excipients such as starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The present compositions, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

The present compositions can take the form of solutions, suspensions, emulsion, tablets, pills, pellets, capsules, capsules containing liquids, powders, sustained-release formulations, suppositories, emulsions, aerosols, sprays, suspensions, or any other form suitable for use. In one embodiment, the pharmaceutically acceptable vehicle is a capsule (see e.g., U.S. Pat. No. 5,698,155). Other examples of suitable pharmaceutical vehicles are described in "Remington's Pharmaceutical Sciences" by A. R. Gennaro.

In a preferred embodiment, the crystalline polymorph or amorphous form of a Compound of the Invention is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, a crystalline polymorph or amorphous form of a Compound of the Invention for intravenous administration is a solution in sterile isotonic aqueous buffer. Where necessary, the compositions may also include a solubilizing agent. Compositions for intravenous administration may optionally include a local anesthetic such as lidocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the crystalline polymorph or amorphous form of a Compound of the Invention is to be administered by infusion, it can be dispensed, for example, with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the compound of the invention is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

It is preferred that the compositions of the invention be administered orally. Compositions for oral delivery may be in the form of tablets, lozenges, aqueous or oily suspensions, granules, powders, emulsions, capsules, syrups, or elixirs, for example. Orally administered compositions may contain one or more optionally agents, for example, sweetening agents such as fructose, aspartame or saccharin; flavoring agents such as peppermint, oil of wintergreen, or cherry; coloring agents; and preserving agents, to provide a pharmaceutically palatable preparation. Moreover, where in tablet or pill form, the compositions may be coated to delay disintegration and absorption in the gastrointestinal tract thereby providing a sustained action over an extended period of time. Selectively permeable membranes surrounding an osmotically active driving compound are also suitable for orally administered crystalline polymorph or amorphous form of a Compound of the Invention. In these later platforms, fluid from the environment surrounding the capsule is imbibed by the driving compound, which swells to displace the agent or agent composition through an aperture. These delivery platforms can provide an essentially zero order delivery profile as opposed to the spiked profiles of immediate release formulations. A time delay material such as glycerol monostearate or glycerol stearate may also be used. Oral compositions can include standard vehicles such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Such vehicles are preferably of pharmaceutical grade.

The amount of a crystalline polymorph or amorphous form of a Compound of the Invention that will be effective in the treatment of a particular disorder or condition disclosed herein will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, in vitro or in vivo assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the compositions will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. However, suitable dosage ranges for oral administration are generally about 0.001 milligram to 1000 milligrams of a compound of the invention per kilogram body weight. In specific preferred embodiments of the invention, the oral dose is 0.01 milligram to 500 milligrams per kilogram body weight, more preferably 0.1 milligram to 100 milligrams per kilogram body weight, more preferably 0.5 milligram to 50 milligrams per kilogram body weight, and yet more preferably 1 milligram to 10 milligrams per kilogram body weight. In a most preferred

embodiment, the oral dose is 1 milligram of a crystalline polymorph or amorphous form of a Compound of the Invention per kilogram body weight. The dosage amounts described herein refer to total amounts administered; that is, if more than one compound of the invention is administered, the preferred dosages correspond to the total amount of the compounds of the invention administered. Oral compositions preferably contain 10% to 95% active ingredient by weight.

Suitable dosage ranges for intravenous (i.v.) administration are 0.001 milligram to 1000 milligrams per kilogram body weight, 0.1 milligram to 100 milligrams per kilogram body weight, and 1 milligram to 10 milligrams per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Suppositories generally contain 0.01 milligram to 50 milligrams of a compound of the invention per kilogram body weight and comprise active ingredient in the range of 0.5% to 10% by weight. Recommended dosages for intradermal, intramuscular, intraperitoneal, subcutaneous, epidural, sublingual, intracerebral, intravaginal, transdermal administration or administration by inhalation are in the range of 0.001 milligram to 1000 milligrams per kilogram of body weight. Suitable doses of the compounds of the invention for topical administration are in the range of 0.001 milligram to 1 milligram, depending on the area to which the compound is administered. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems. Such animal models and systems are well known in the art.

The invention also provides pharmaceutical packs or kits comprising one or more containers filled with one or more crystalline polymorph or amorphous form of a Compound of the Invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In a certain embodiment, the kit contains more than one crystalline polymorph or amorphous form of a Compound of the Invention.

The crystalline polymorph or amorphous form of a Compound of the Invention is preferably assayed in vitro and in vivo, for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays can be used to determine whether administration of a specific compound of the invention or a combination of compounds of the invention is preferred for lowering fatty acid synthesis. The compounds of the invention may also be demonstrated to be effective and safe using animal model systems.

Other methods will be known to the skilled artisan and are within the scope of the invention.

6.4. General Synthesis of the Compounds of the Invention

The 18-membered macrocycles and analogs thereof are produced by fermentation. Cultivation of *Dactylosporangium aurantiacum* subspecies *hamdenensis* AB 718C-41 NRRL 18085 for the production of the tiacumicins is carried out in a medium containing carbon sources, inorganic salts and other organic ingredients with one or more absorbents under proper aeration conditions and mixing in a sterile environment.

The microorganism to produce the active antibacterial agents was identified as belonging to the family Actinoplanaceae, genus *Dactylosporangium* (*J. Antibiotics,* 1987, 40: 567-574 and U.S. Pat. No. 4,918,174). It has been designated *Dactylasporangium aurantiacum* subspecies *hamdenensis* 718C-41. The subculture was obtained from the ARS Patent Collection of the Northern Regional Research Center, United States Department of Agriculture, 1815 North University Street, Peoria, Ill. 61604, U.S.A., where it was assigned accession number NRRL 18085. The characteristics of strain AB 718C-41 are given in the *Journal of Antibiotics,* 1987, 40: 567-574 and U.S. Pat. No. 4,918,174.

This invention encompasses the composition of novel antibiotic agents, Tiacumicins, by submerged aerobic fermentation of the microorganism *Dactylosporangium aurantiacum* subspecies *hamdenensis*. The production method is disclosed in WO 2004/014295 A2, which is hereby incorporated by reference.

7. EXAMPLES

7.1. Preparation of the Crude Mixtures of Tiacumicins and the Subsequent Crystallization of Certain Polymorphs of the Mixtures

In an illustrative embodiment, a mixture of tiacumicins containing the Compound of Formula I is prepared by a process comprising:

(i) culturing a microorganism in a nutrient medium to accumulate the mixture in the nutrient medium; and

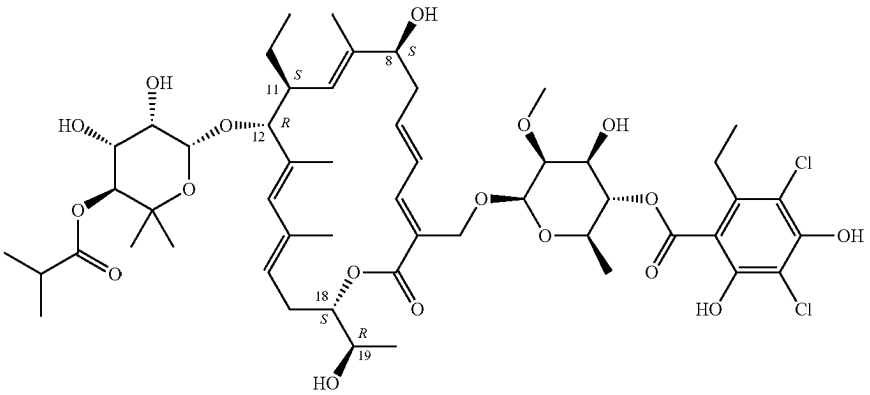
(ii) isolating the mixture from the nutrient medium; wherein the nutrient medium comprises an adsorbent to adsorb the mixture.

The nutrient medium preferably comprises from about 0.5 to about 15% of the adsorbent by weight. The absorbent is preferably an adsorbent resin. More preferably, the adsorbent resin is Amberlite®, XAD16, XAD16HP, XAD2, XAD714P, XAD1180, XAD1600, IRC50, or Duolite® XAD761. The microorganism is preferably *Dactylosporangium aurantiacum* subspecies *hamdenensis*. The nutrient medium comprises the following combination based on weight: from about 0.2% to about 10% of glucose, from about 0.02% to about 0.5% of K_2HPO_4, from about 0.02% to about 0.5% of $MgSO_4.7H_2O$, from about 0.01% to about 0.3% of KCl, from about 0.1% to about 2% of $CaCO_3$, from about 0.05% to about 2% of casamino acid, from about 0.05% to about 2% of yeast extract, and from about 0.5% to about 15% of YAD-16 resin. The culturing step is preferably conducted at a temperature from about 25° C. to about 35° C. and at a pH from about 6.0 to about 8.0.

Upon completion of fermentation, the solid mass (including the adsorbent resin) is separated from the broth by sieving. The solid mass is eluted with organic solvents such as, for example, ethyl acetate then concentrated under reduced pressure.

7.2. Structure of R-Tiacumicin B

The structure of the R-Tiacumicin B (the major most active component) is shown below in Formula I. The X-ray crystal structure of the R-Tiacumicin B was obtained as a colorless, parallelepiped-shaped crystal (0.08×0.14×0.22 mm) grown in aqueous methanol. This x-ray structure confirms the structure shown below. The official chemical name is 3-[[[6-Deoxy-4-O-(3,5-dichloro-2-ethyl-4,6-dihydroxybenzoyl)-2-O-methyl-β-3-D-mannopyranosyl]oxy]-methyl]-12(R)-[[6-deoxy-5-C-methyl-4-O-(2-methyl-1-oxopropyl)-β-D-lyxo-hexopyranosyl]oxy]-11(S)-ethyl-8(S)-hydroxy-18(S)-(1(R)-hydroxyethyl)-9,13,15-trimethyloxacyclooctadeca-3,5,9,13,15-pentaene-2-one.



7.2.1 Analytical Data of R-Tiacumicin B

The analytical data of R-Tiacumicin B (which is almost entirely (i.e., >90%) R-Tiacumicin).

mp 166-169° C. (white needle from isopropanol);

$[\alpha]_D^{20}$ −6.9 (c 2.0, MeOH);

MS m/z (ESI) 1079.7 $(M+Na)^+$;

^{1}H NMR (400 MHz, CD_3OD) δ 7.21 (d, 1H), 6.59 (dd, 1H), 5.95 (ddd, 1H), 5.83 (br s, 1H), 5.57 (t, 1H), 5.13 (br d, 1H), 5.09 (t, 1H), 5.02 (d, 1H), 4.71 (m, 1H), 4.71 (br s, 1H), 4.64 (br s, 1H), 4.61 (d, 1H), 4.42 (d, 1H), 4.23 (m, 1H), 4.02 (pentet, 1H), 3.92 (dd, 1H), 3.73 (m, 2H), 3.70 (d, 1H), 3.56 (s, 3H), 3.52-3.56 (m, 2H), 2.92 (m, 2H), 2.64-2.76 (m, 3H), 2.59 (heptet, 1H), 2.49 (ddd, 1H), 2.42 (ddd, 1H), 2.01 (dq, 1H), 1.81 (s, 3H), 1.76 (s, 3H), 1.65 (s, 3H), 1.35 (d, 3H), 1.29 (m, 1H) 1.20 (t, 3H), 1.19 (d, 3H), 1.17 (d, 3H), 1.16 (d, 3H), 1.14 (s, 3H), 1.12 (s, 3H), 0.87 (t, 3H);

^{13}C NMR (100 MHz, CD_3OD) δ 178.4, 169.7, 169.1, 154.6, 153.9, 146.2, 143.7, 141.9, 137.1, 137.0, 136.4, 134.6, 128.5, 126.9, 125.6, 124.6, 114.8, 112.8, 108.8, 102.3, 97.2, 94.3, 82.5, 78.6, 76.9, 75.9, 74.5, 73.5, 73.2, 72.8, 71.6, 70.5, 68.3, 63.9, 62.2, 42.5, 37.3, 35.4, 28.7, 28.3, 26.9, 26.4, 20.3, 19.6, 19.2, 18.7, 18.2, 17.6, 15.5, 14.6, 14.0, 114.

7.3. Preparation of a First Polymorph of R-Tiacumicin B

Another illustrative embodiment of the invention comprises a process for producing a polymorph of a Compound of Formula I from a mixture of tiacumicins comprising the steps of:

a) dissolving a crude mixture of tiacumicins containing from about 76% to about 100% of a Compound of Formula I in a minimum amount of solution comprising methanol, water, acetonitrile, acetic acid, or isopropyl alcohol mixtures thereof;

b) allowing the solution of a) to evaporate while standing at room temperature (e.g., about 22° C.) for 3 to 7 days to precipitate a first polymorph of a Compound of Formula I; and

c) separating the polymorph from the solution by techniques known in the art.

7.3.1. Illustrative Example 1 of the Preparation of a Polymorph of R-Tiacumicin B

After the fermentation process as described for example in Section 7.1, the crude material was purified by reverse phase chromatography using a Biotage Flash 75L system containing a 1.2 kg, Biotage KP-C18-HS silica column, eluted with 70:30:1, MeOH/H_2O/AcOH. The collected fractions containing 75-80% of Compound of Formula I were combined and concentrated to one-third of the original volume to produce a precipitate. The precipitate is filtered and washed with water. The solid was dried under high vacuum to afford an off-white powder. HPLC analysis showed the powder contains about 78% of Compound of Formula I as a major product and a mixture of tiacumicins as the minor component.

The mixture of tiacumicins containing about 78% of Compound of Formula I (i.e., 50 mg) was dissolved in 2 mL of methanol followed by addition of 1 mL of water. The solution was allowed to evaporate, while standing at room temperature for 7 days to produce a crystalline precipitate. The crystal is separated from the solution by filtration. After methanol/water recrystallization, the crystals contain about 90% of Compound of Formula I based on HPLC.

7.3.2. Illustrative Example 2 of the Preparation of a Polymorph of R-Tiacumicin

After the fermentation process as described for example in Section 7.1, the crude material was purified by reverse phase chromatography using a Biotage Flash 150 system containing a 3.75 kg, Biotage KP-C18-HS silica column, eluted with 52:48:1, EtOH/H_2O/AcOH. The collected fractions containing about 80-88% of Compound of Formula I were combined and concentrated to one-third the original volume to produce a precipitate. The precipitate was filtered and washed with water. The solid was dried under high vacuum. HPLC analysis showed the powder contains 85.4% of Compound of Formula I as a major product and a mixture of tiacumicins as the minor component.

The mixture containing about 85% of Compound of Formula I (i.e., 1000 mg) was dissolved in 20 mL of a mixture of methanol and water at ratios 1:1 methanol water. The solution was allowed to evaporate/stand at room temperature for 3 days to produce a polymorph crystalline precipitate. The crystal was separated from the solution by filtration.

The composition obtained is a mixture containing a first polymorph of a Compound of Formula I, and at least one of the tiacumicin compounds based on HPLC analysis. The composition has a melting point of 165-169° C.

7.3.3. Illustrative Example 3 of the Preparation of a Polymorph of R-Tiacumicin

After the fermentation process as described for example in Section 7.1, the crude material was purified by reverse phase chromatography using a Biotage Flash 75L system containing a 1.2 kg, Biotage KP-C18-HS silica column, eluted with MeOH/H_2O/AcOH 67:33:4 to 70:30:1. The collected fractions containing >90% of Compound of Formula I was combined and concentrated to one-third volume. The precipitate

was filtered and washed with water. The solid was dried under high vacuum, MPLC analysis showed the powder contains 94.0% of Compound of Formula I.

The solid was tested by X-ray diffraction (XRD) and Differential Scanning Calorimetry (DSC) (See FIG. **2**). The X-ray diffraction of the solid shows peaks at angles 2θ of 7.7°, 15.0°, and 18.8°±0.1 indicating the solid is the form of a first polymorph of a Compound of Formula I. The DSC plot shows an endothermic curve starting at about at 169° C. and peak at 177° C.

7.3.4. Illustrative Example 4 of the Preparation of a Polymorph of R-Tiacumicin

After the fermentation process as described for example in Section 7.1, the crude material was purified by reverse phase chromatography using a Biotage Flash 75L system containing a 1.2 kg, Biotage KP-C18-HS silica column, eluted with 52:48:1, EtOH/H_2O/AcOH. The collected fractions containing >90% of Compound of Formula I were combined, one-third volume of water was added and left at room temperature overnight. The precipitate was filtered and washed with water. The solid was dried under high vacuum. HPLC analysis showed the powder contains 94.7% of Compound of Formula I.

The powder containing 94.7% of Compound of Formula I (i.e., 98 mg) was dissolved in 3 mL of methanol and then 1 mL of water was added. The solution was allowed to evaporate and stand at room temperature for 7 days to produce a crystalline precipitate. The crystals were separated from the solution by filtration and washed with methanol/water 3:1. The crystals were analyzed by X-ray diffraction.

Composition of the precipitate is a mixture comprising a Compound of Formula I based on HPLC analysis with a melting point of 166-169° C.

7.3.5. Illustrative Example 5 of the Preparation of a Polymorph of R-Tiacumicin

After the fermentation process as described for example in Section 7.1, the mixture was purified on a column, and a 0.06 gm of a mixture of tiacumicins was dissolved in 16 mL of methanol and 4 mL of water in a 20 mL vial. The vial is covered with parafilm, and pinholes were punched through. The covered vial is placed in a desiccator and stored at room temperature for ten days. Parafilm cover is then removed, and the vial is returned to desiccator. Crystalline material is produced within three to five days after the parafilm is removed. The crystalline material is washed with a solution of methanol and water and the Compound of Formula I was isolated in 75.6%.

X-ray powder diffraction pattern of the crystalline material is shown in FIG. **3** included 2θ of 7.7°, 15.0°, and 18.0°.

7.3.6. Illustrative Example 6 of the Preparation of a Polymorph of R-Tiacumicin

Preparation of a Polymorph from Isopropanol

After the fermentation process as described for example in Section 7.1, the crude material was purified by reverse phase chromatography using a Biotage Flash 150 system containing a 3.75 kg, Biotage KP-C18-HS silica column, eluted with 52:48:1, EtOH/H_2O/AcOH. The collected fractions containing 80-88% of Compound of Formula I were combined and concentrated to one-third of the original volume to produce a precipitate. The precipitate was filtered and washed with water. The solid was dried under high vacuum. HPLC analysis showed the powder contains 85.4% of Compound of Formula I.

The powder containing 85.4% Compound of Formula I (i.e., 2000 mg) was dissolved in 900 mL of isopropanol. The solution was heated to increase solubility and then filtered to remove insoluble materials. The clear solution was allowed to evaporate/stand at room temperature for 14 days to produce a crystalline precipitate. The crystal is separated from the solution by filtration.

Composition of the precipitate is a mixture comprising Compound of Formula I and at least one of other related substances based on HPLC analysis with mp of 163-165° C.

X-ray diffraction of the precipitate shows peaks at angles 2θ of 7.6° and 15.4°.

7.3.7. Illustrative Example 7 of the Preparation of a Polymorph of R-Tiacumicin

After the fermentation process as described for example in Section 7.1, and column purification, a mixture of Compound of Formula I, >90%, 15 g) was dissolved in minimum amount of methanol (from about 20 mL to about 30 mL), the solution was triturated with isopropanol (~100 mL) to produce a polymorph. The solid is separated from the solution by filtration with melting point of 165-168° C.

The XRD diagram shows a distinct polymorph pattern comprising 2 theta values of 7.5°, 15.2°, 15.7°, 18.6° 18.7°.

7.3.8. Illustrative Example 5 of the Preparation of a Polymorph of R-Tiacumicin

Preparation of a Polymorph from Acetonitrile

The mixture of tiacumicins obtained as described above and (85-44% of Compound of Formula I, 1000 mg) was dissolved in 30 mL of acetonitrile. The solution was allowed to evaporate and stand at room temperature for 12 days to produce a crystalline precipitate. The crystal is separated from the solution by filtration, and exhibits a melting point of 165-169° C.

The XRD diagram of this crystal shows the pattern of a polymorph comprising 2 theta values of 7.8°, 15.1°, 18.8°.

7.4. Preparation of Other Polymorphs of R-Tiacumicin

Another illustrative embodiment of the invention comprises a process for producing a polymorph of a Compound of Formula I comprising the steps of:

a) dissolving crude mixture of tiacumicins containing from about 78 to about 100% of a Compound of Formula I in a minimum amount of ethyl acetate;

b) allowing the solution to evaporate and stand at room temperature for 3 to 7 days to precipitate a polymorph; and

c) separating polymorph from the solution

7.4.1. Illustrative Example 1 of the Preparation of a Polymorph of R-Tiacumicin

Preparation of Polymorph from Ethyl Acetate

After the fermentation process as described for example in Section 7.1, the crude material was purified by reverse phase chromatography using a Biotage Flash 150 system containing a 3.75 kg, Biotage KP-C18-HS silica column, eluted with 52:48:1, EtOH/H_2O/AcOH. The collected fractions containing 70-88% of Compound of Formula I was combined and concentrated to one-third volume to produce a precipitate. The precipitate is filtered and washed with water. The solid was dried under high vacuum, HPLC analysis showed the powder contains 85.4% of Compound of Formula I.

This crude tiacumicin mixture (1000 mg) was then dissolved in 30 mL of ethyl acetate. The solution was allowed to evaporate and stand at room temperature for 12 days to produce a crystalline precipitate of Polymorph B of the Compound of Formula I. The crystals were separated from the solution by filtration. The crystals have a melting point of about 153-156° C., which confirm a different polymorphic form from the first polymorph.

7.4.2. Illustrative Example 2 of the Preparation of a Polymorph of R-Tiacumicin

Preparation of a Polymorph from Methanol and Isopropanol.

After the fermentation process as described for example in Section 7.1, six different batches of crude material of varying amounts of Compound of Formula I were combined such that the combination has an average of 91% of Compound of Formula I. The combination was dissolved in methanol and concentrated by rotary evaporation. The concentrated solution is then mixed with isopropanol, filtered, and dried by vacuum to produce a white powder with a melting point of 156-160° C.

X-ray powder diffraction of the white powder comprises 2θ values of 7.5°, 15.4°, and 18.7°.

7.4.3. Illustrative Example 3 of the Preparation of a Polymorph of R-Tiacumicin

Preparation of Polymorph B From Chloroform

After the fermentation process as described for example in Section 7.1, a crude material of tiacumicins containing Compound of Formula I was dissolved in chloroform and concentrated by evaporation at room temperature to produce a solid with a melting point of 156-160° C.

7.4.4. Illustrative Example 4 of the Preparation of a Polymorph of R-Tiacumicin

Preparation of a Polymorphic Form from Acetone

After the fermentation process as described for example in Section 7.1, a crude material of tiacumicins containing Compound of Formula I was dissolved in acetone and concentrated by evaporation at room temperature to produce a solid with a melting point of 156-160° C.

7.5. Preparation of Amorphous Forms of Compound of Formula I

Preparation of Amorphous Mixture of Tiacumicins

The amorphous mixture of tiacumicins was obtained after column purification without any further processing steps. Alternatively, chloroform or acetone may be added to the mixture of tiacumicins and the solvent is evaporated to form the amorphous product.

X-ray powder diffraction of the product exhibits no defined diffraction peaks.

8. EXPERIMENTAL DATA

8.1. Polymorph Experimental Data

A first polymorph of a Compound of a Compound of Formula I is characterized by Differential Scanning Calorimetry ("DSC") and powder X-Ray Diffraction ("XRD").

The DSC plot of the polymorph shows an endothermic curve at 177° C.

The XRD diagram (reported in FIG. **1**) shows peaks comprising at diffraction angles 2θ of 7.7°, 15.0°, 18.8°. The XRD was analyzed with a Phillips powder Diffractometer by scanning from 20 to 70 degrees two-theta at 1.0 degree per minute using Cu K-alpha radiation, at 35 kV and 20 ma. The instrumental error (variant) is 0.04 (2 theta value).

The melting point of the mixtures containing various amounts of Compound of Formula I is summarized in Table 1. All of the products with at least 85% of a Compound of Formula I in the form of a polymorph appear to have a melting point in the range of 163-169° C. measured by Melting Point apparatus, MEL-TEMP 1001.

TABLE 1

Melting point of polymorph mixtures in different solvent conditions

No.	Compound of Formula I Content (%) of the crystalline material	Mp (° C.)	Crystallization Solvent
1	85	165-169	MeOH/Water
2	85	163-165	Isopropanol
3	85	164-168	Acetonitrile
4	90	165-168	MeOH/Isopropanol
5	94	166-169	MeOH/Water
6	95	166-169	MeOH/Water
7	98	163-164	MeOH/Isopropanol

Composition of the a polymorphic crystal from a mixture comprising Compound of Formula I and optionally at least on compound that is a mixture of tiacumicins based on HPLC analysis with a melting point of 166-169° C.

X-ray diffraction of a polymorphic crystal shows characteristic peaks at angles 2θ of 7.8°, 15.0°, 18.8°, and 23.9°. Table 2 is a listing of the obtained X-ray diffraction peaks for first polymorph of R-Tiacumicin from Experiment 7.2.2.

TABLE 2

X-ray diffraction peaks for a First Polymorph from Experiment 7.3.2.

Two-Theta	Relative Intesity
3.3568	44.0000
3.4400	47.0000
7.7815	112.0000
10.1575	32.0000
13.6023	21.0000
15.0951	139.0000
17.0178	18.0000
18.8458	36.0000
19.3771	9.0000
20.0300	16.0000
20.4842	10.0000
23.9280	136.0000
24.8338	10.0000
25.0889	19.0000
25.7256	10.0000
30.9126	75.0000
31.9970	10.0000
34.4507	30.0000

Table 3 is a listing of the obtained X-ray diffraction peaks for Polymorph from Experiment 7.3.6.

TABLE 3

X-ray diffraction peaks for a Polymorph from Experiment 7.3.6.

Two-Theta	Relative Intensity
3.2978	41.0000
7.5615	400.0000
9.9482	21.0000
15.4289	31.0000
22.0360	20.0000
22.5361	20.0000
24.9507	12.0000
29.5886	10.0000
34.8526	19.0000
37.7092	17.0000
40.4361	13.0000
42.2446	18.0000

8.2 Second Polymorph of R-Tiacumicin Experimental Data

A second polymorph of Compound of Formula I is also characterized by Differential Scanning Calorimetry (DSC) and powder X-Ray Diffraction (XRD).

The DSC plot of polymorph B shows an endothermic curve at 158° C. The XRD diagram shows peaks comprising at the values of the diffraction angles 2θ of 7.6°, 15.4° and 18.8°. Polymorph B has a melting point in the range of 153-156° C. measured by Melting Point apparatus, MEL-TEMP 1001.

It is believed that crystalline polymorphic forms of Compounds of Formula I other than the above-discussed A and B exist and are disclosed herein. These crystalline polymorphic forms, including A and B, and the amorphous form or mixtures thereof contain varying amounts of Compound of Formula I and in certain cases mixtures of tiacumicins can be advantageously used in the production of medicinal preparations having antibiotic activity.

X-ray powder diffraction of the crystals is shown in FIG. **3** with peaks at angles 2θ of 7.5°, 15.7°, and 18.9°±0.04 indicating the presence of Polymorph B.

The DSC plot of Polymorph B shows an endothermic curve starting at about 150° C. and peak at 158° C.

Table 4 is a summary of the various data that was isolated for illustrative crystallization lots.

Formula I

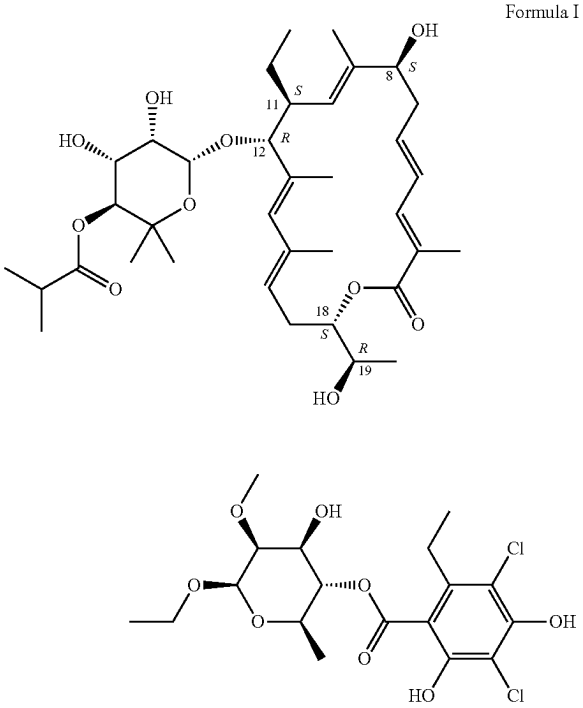


TABLE 4

Data Summarizing Various Lots

No.	Compound of Formula I Content (%)	Mp (° C.)	DSC (° C.) Peak	XRD (2 theta)	Crystallization Solvent
1	76.3	155-158		7.7, 15.0, 18.8,	MeOH/Water
2	85.3	159-164	180	7.8, 14.9, 18.8,	MeOH/Water
3	85.4	163-165		7.6, 15.4	Iso-propanol (IPA)
4	85.4	164-168		7.9, 15.0, 18.8	Acetonitrile
5	85.4	153-156		7.5, 15.7, 18.9	EtOAc
6	90	165-168		7.5, 15.2, 15.7, 18.6	MeOH/Isopropanol
7	97.2	160-163	177	7.4, 15.4, 18.7	IPA
8	94.0	166-169	177	7.6, 15.1, 18.6	MeOH/Water
9	97.2	167-173	187	7.8, 14.8, 18.8	MeOH/Water
10	96.7		160	7.5, 15.4, 18.8	EtOAc
11	98.3	163-164	178	7.7, 15.0, 18.8	MeOH/IPA

The present invention is not to be limited in scope by the specific embodiments disclosed in the examples which are intended as illustrations of a few aspects of the invention and any embodiments which are functionally equivalent are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art and are intended to fall within the appended claims.

A number of references have been cited, the entire disclosures of which are incorporated herein by reference.

What is claimed is:

1. A pharmaceutical composition comprising a therapeutically effective amount of a polymorphic form of a compound of Formula I:

wherein the polymorphic form of a compound of Formula I is characterized by a powder x-ray diffraction pattern wherein said x-ray diffraction pattern comprises peaks at diffraction angles 2θ of 7.7°, 15.0°, and 18.8°±0.2 as said peaks are set forth in FIG. **1**.

2. The pharmaceutical composition of claim **1**, wherein the therapeutically effective amount of a polymorphic form of a compound of Formula I comprises about 0.001 mg to about 1000 mg.

3. The pharmaceutical composition of claim **1**, wherein the therapeutically effective amount of a polymorphic form of a compound of Formula I comprises about 0.01 mg to about 500 mg.

4. The pharmaceutical composition of claim **1**, wherein the therapeutically effective amount of a polymorphic form of a compound of Formula I comprises about 0.1 mg to about 100 mg.

5. The pharmaceutical composition of claim **1**, wherein the therapeutically effective amount of a polymorphic form of a compound of Formula I comprises about 0.5 mg to about 50 mg.

6. The pharmaceutical composition of claim **1** suitable for oral administration.

7. The pharmaceutical composition of claim **1** suitable for topical administration.

8. The pharmaceutical composition of claim **1**, wherein the polymorphic form of a compound of Formula I is a lyophilized powder.

9. The pharmaceutical composition of claim **8**, further comprising a pharmaceutically acceptable carrier.

10. The pharmaceutical composition of claim **1**, wherein the polymorphic form of the compound of Formula I is characterized by a DSC endotherm in the range of about 174° C. to about 186° C.

11. The pharmaceutical composition of claim **1**, wherein the polymorphic form of the compound of Formula I is characterized by:

(i) a powder x-ray diffraction pattern wherein said x-ray diffraction pattern comprises peaks at diffraction angles 2θ of 7.7°, 15.0°, and 18.8°±0.2 as said peaks are set forth in FIG. **1**; and

(ii) a DSC endotherm in the range of about 174° C. to about 186° C.

12. The pharmaceutical composition of claim **1**, wherein the polymorphic form of the compound of Formula I is characterized by a powder X-ray diffraction pattern as set forth in FIG. **1**.

13. The pharmaceutical composition of claim **1**, wherein the polymorphic form of the compound of Formula I is present in the composition in about 75 wt. % to about 99.99 wt. %.

14. The pharmaceutical composition of claim **1**, wherein the polymorphic form of the compound of Formula I is present in the composition in at least about 85 wt. %.

15. The pharmaceutical composition of claim **1**, wherein the polymorphic form of the compound of Formula I is present in the composition in at least about 90 wt. %.

16. The pharmaceutical composition of claim **1**, wherein the polymorphic form of the compound of Formula I is present in the composition in at least about 95 wt. %.

17. The pharmaceutical composition of claim **1**, wherein the polymorphic form of the compound of Formula I is present in the composition in at least about 99 wt. %.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

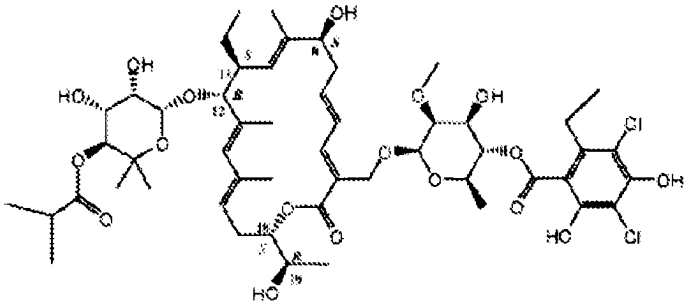
CERTIFICATE OF CORRECTION

PATENT NO. : 7,863,249 B2
APPLICATION NO. : 12/101552
DATED : January 4, 2011
INVENTOR(S) : Yu-Hung Chiu et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In column 28, line 9, delete "FIG. 3 included 2θ of 7.7°, 15.0°, and 18.0°" and insert --FIG. 1 included 2θ of 7.7°, 15.0°, and 18.8°--

In claim 1 (column 32, lines 1-52), Formula I should appear as follows:



Signed and Sealed this
Eighth Day of March, 2011

David J. Kappos

David J. Kappos
Director of the United States Patent and Trademark Office

EXHIBIT 5

US008859510B2

(12) **United States Patent**
Chiu et al.

(10) **Patent No.: US 8,859,510 B2**
(45) **Date of Patent: *Oct. 14, 2014**

(54) **MACROCYCLIC POLYMORPHS, COMPOSITIONS COMPRISING SUCH POLYMORPHS, AND METHODS OF USE AND MANUFACTURE THEREOF**

(75) Inventors: **Yu-Hung Chiu**, San Diego, CA (US); **Tessie Mary Che**, San Diego, CA (US); **Alex Romero**, Oakland, CA (US); **Yoshi Ichikawa**, San Diego, CA (US); **Youe-Kong Shue**, San Diego, CA (US)

(73) Assignee: **Optimer Pharmaceuticals, Inc.**, Lexington, MA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 763 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **12/523,790**

(22) PCT Filed: **Jan. 22, 2008**

(86) PCT No.: **PCT/US2008/000735**

§ 371 (c)(1),
(2), (4) Date: **Sep. 4, 2009**

(87) PCT Pub. No.: **WO2008/091554**

PCT Pub. Date: **Jul. 31, 2008**

(65) **Prior Publication Data**

US 2010/0081800 A1 Apr. 1, 2010

Related U.S. Application Data

(60) Provisional application No. 60/881,950, filed on Jan. 22, 2007.

(51) **Int. Cl.**
A61K 31/7048 (2006.01)
C07D 407/14 (2006.01)
C07H 17/08 (2006.01)

(52) **U.S. Cl.**
CPC ***C07H 17/08*** (2013.01); ***C07D 407/14*** (2013.01)
USPC **514/28**; 536/7.1

(58) **Field of Classification Search**
USPC 514/28
See application file for complete search history.

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(Continued)

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(57) **ABSTRACT**

The invention relates to novel forms of compounds displaying broad spectrum antibiotic activity, especially crystalline polymorphic forms and amorphous forms of such compounds, compositions comprising such crystalline polymorphic forms and amorphous forms of such compounds, processes for manufacture and use thereof. The compounds and compositions of the invention are useful in the pharmaceutical industry, for example, in the treatment or prevention of diseases or disorders associated with the use of antibiotics, chemotherapies, or antiviral therapies, including, but not limited to, colitis, for example, pseudo-membranous colitis; antibiotic associated diarrhea; and infections due to *Clostridium difficile* ("*C. difficile*"), *Clostridium perfringens* ("*C. perfringens*"), *Staphylococcus* species, for example, methicillin-resistant *Staphylococcus*, or *Enterococcus* including Vancomycin-resistant *enterococci.*

13 Claims, 3 Drawing Sheets

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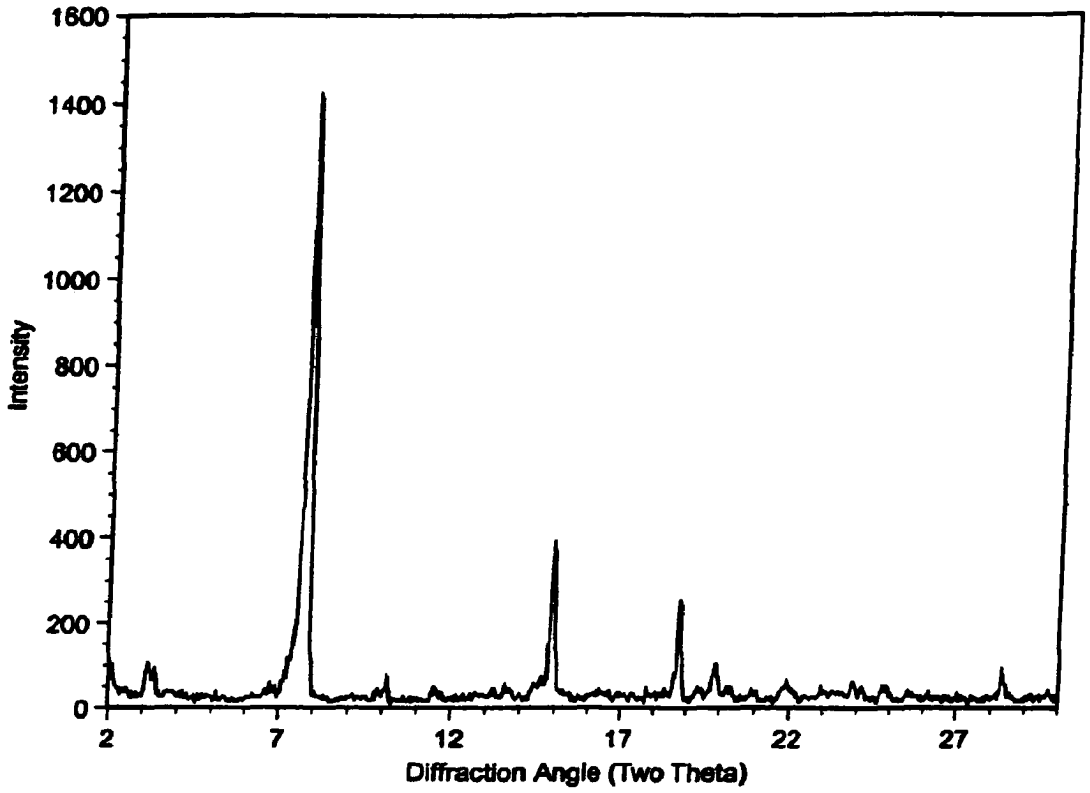
1600
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Diffraction Angle (Two Theta)

Figure 1

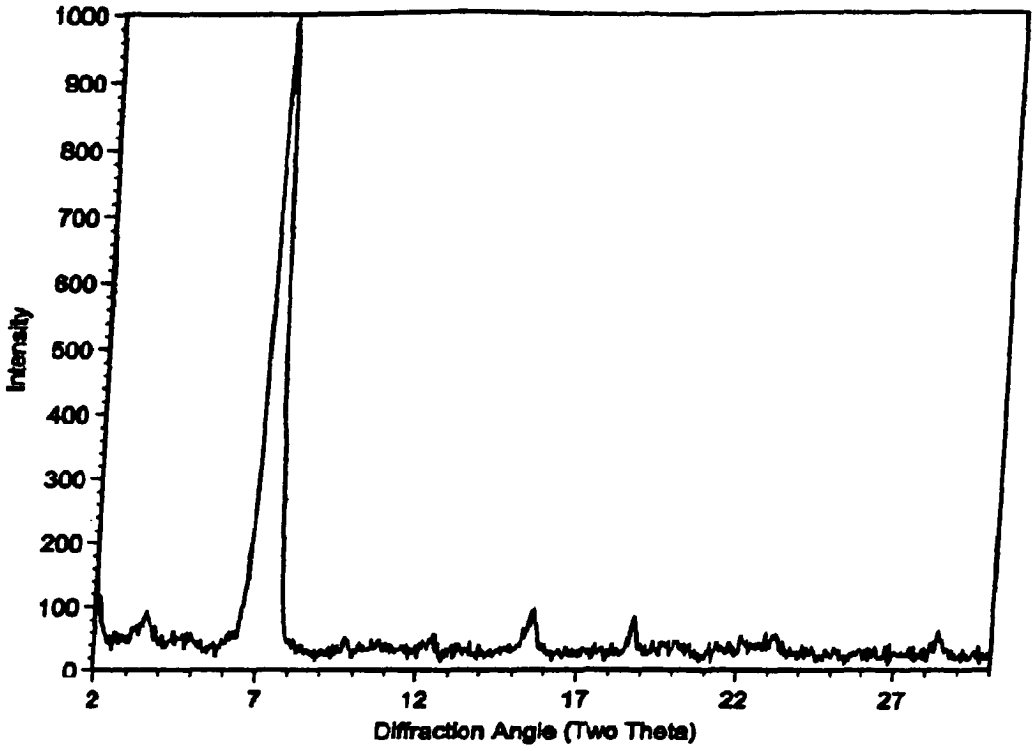
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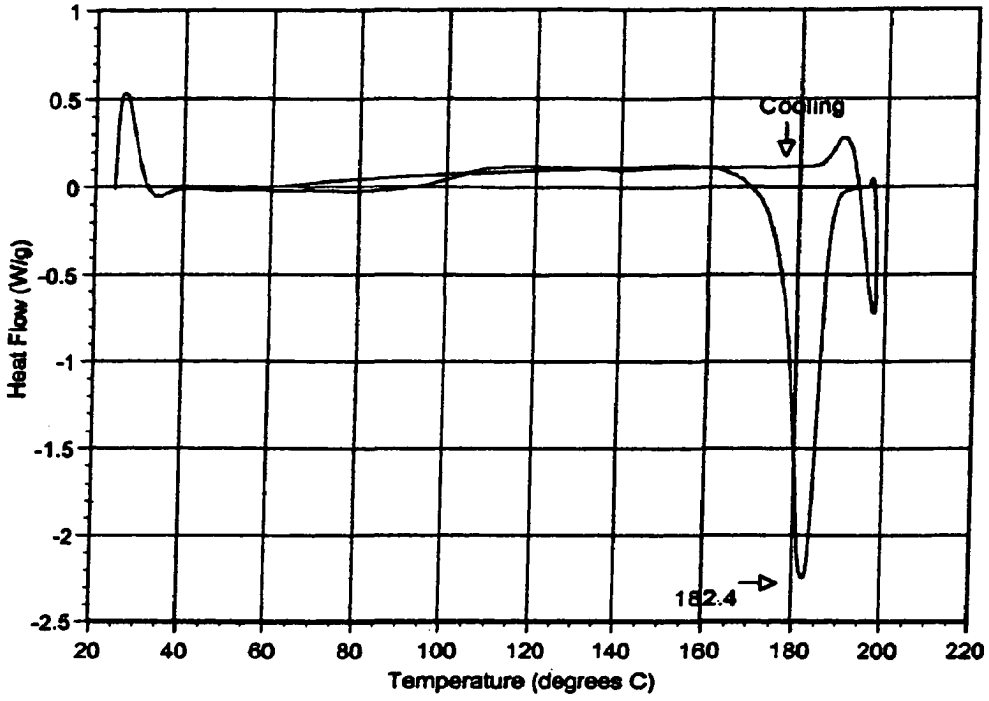
Figure 2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Heat Flow (W/g)
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40
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80
100
120
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160
180
200
220
Temperature (degrees C)

Figure 3

MACROCYCLIC POLYMORPHS, COMPOSITIONS COMPRISING SUCH POLYMORPHS, AND METHODS OF USE AND MANUFACTURE THEREOF

1. RELATED APPLICATIONS

This application is a U.S. National Phase Application of International Application Number PCT/US2008/000735 filed Jan. 22, 2008, which claims the benefit of U.S. Provisional Patent Application No. 60/881,950, filed Jan. 22, 2007, each of which is incorporated by reference in its entirety.

2. FIELD OF THE INVENTION

The invention encompasses novel forms of compounds displaying broad spectrum antibiotic activity, especially crystalline polymorphic forms and amorphous forms of such compounds, compositions comprising such crystalline polymorphic forms and amorphous forms of such compounds, processes for manufacture and use thereof. The compounds and compositions of the invention are useful in the medical and pharmaceutical industry, for example, in the treatment or prevention of diseases or disorders associated with the use of antibiotics, chemotherapies, or antiviral therapies, including, but not limited to, colitis, for example, pseudo-membranous colitis; antibiotic associated diarrhea; and infections due to *Clostridium difficile* ("*C. difficile*"), *Clostridium perfringens* ("*C. perfringens*"), *Staphylococcus* species, for example, methicillin-resistant *Staphylococcus*, or *Enterococcus* including Vancomycin-resistant *enterococci.*

3. BACKGROUND OF THE INVENTION

Antibiotic-associated diarrhea ("AAD") diseases are caused by toxin producing strains of *C. difficile, Staphylococcus aureus* ("*S. aureus*") including methicillin-resistant *Staphylococcus aureus* ("MRSA") and *C. perfringens*. AAD represents a major economic burden to the healthcare system that is conservatively estimated at $3-6 billion per year in excess hospital costs in the United States alone.

AAD is a significant problem in hospitals and long-term care facilities. *C. difficile* is the leading cause of AAD in the hospital setting, accounting for approximately 20% of cases of AAD and the majority of cases of antibiotic-associated colitis ("AAC"). The rising incidence of *C. difficile* associated diarrhea ("CDAD") has been attributed to the frequent prescribing of broad-spectrum antibiotics to hospitalized patients.

The tiacumicins are a group of 18-membered macrolide antibiotics originally isolated from the fermentation broth of *Dactylosporangium aurantiacum*. The tiacumicins are effective Gram-positive antibiotics. In particular, tiacumicins, specifically Tiacumicin B, show activity against a variety of bacterial pathogens and in particular against *C. difficile*, a Gram-positive bacterium (*Antimicrob. Agents Chemother.,* 1991, 1108-1111). A purification of tiacumicins was carried out in suitable solvents, wherein tiacumicin B exhibited a

melting point of 143-145° C. (See, e.g., J. E. Hochlowski, et al., *J. Antibiotics*, vol. XL, no. 5, pages 575-588 (1987)).

The polymorphic behavior of a compound can be of crucial importance in pharmacy and pharmacology. Polymorphs are, by definition, crystals of the same molecule having different physical properties as a result of the order of the molecules in the crystal lattice. The differences in physical properties exhibited by polymorphs affect pharmaceutical parameters such as storage stability, compressibility and density (important in formulation and product manufacturing), and dissolution rates (an important factor in determining bio-availability). Differences in stability can result from changes in chemical reactivity (e.g., differential oxidation, such that a dosage form discolors more rapidly when comprised of one polymorph than when comprised of another polymorph) or mechanical changes (e.g., tablets crumble on storage as a kinetically favored polymorph converts to thermodynamically more stable polymorph) or both (e.g., tablets of one polymorph are more susceptible to breakdown at high humidity). As a result of solubility/dissolution differences, in the extreme case, some polymorphic transitions may result in lack of potency or, at the other extreme, toxicity. In addition, the physical properties of a crystal may be important in processing: for example, one polymorph might be more likely to form solvates or might be difficult to filter and wash free of impurities (i.e., particle shape and size distribution might be different between one polymorph relative to the other).

Each pharmaceutical compound has an optimal therapeutic blood concentration and a lethal concentration. The bio-availability of the compound determines the dosage strength in the drug formulation necessary to obtain the ideal blood level. If the drug can crystallize as two or more polymorphs differing in bio-availability, the optimal dose will depend on the polymorph present in the formulation. Some drugs show a narrow margin between therapeutic and lethal concentrations. Thus, it becomes important for both medical and commercial reasons to produce and market the drug in its most thermodynamically stable polymorph, substantially free of other kinetically favored or disfavored polymorphs.

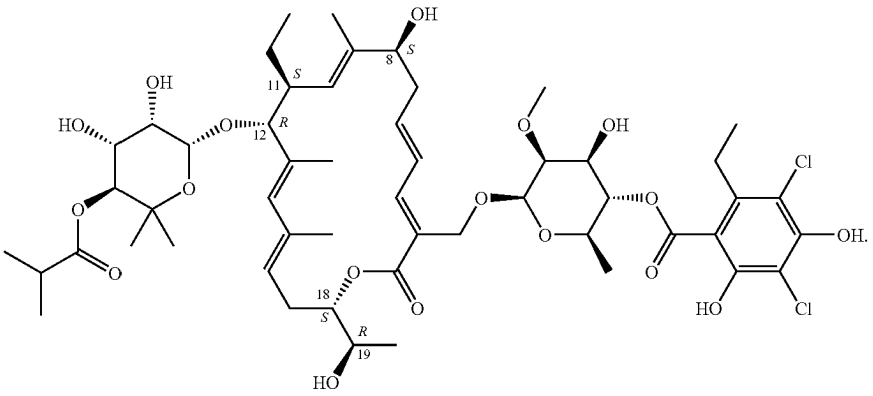
Thus, there is a clear need to develop safe and effective polymorphs of drugs that are efficacious at treating or preventing disorders associated with bacterial pathogens. The present inventors have identified novel crystalline and amorphous forms of 18-membered macrolide compounds that exhibit broad spectrum antibiotic activity.

4. SUMMARY OF THE INVENTION

The invention encompasses novel crystalline and amorphous forms of the macrolide compounds that are useful in treating or preventing bacterial infections and protozoal infections. In an illustrative embodiment, the novel crystalline and amorphous forms of the macrolide compounds of the invention exhibit broad spectrum antibiotic activity. Thus, surprisingly novel crystalline and amorphous forms of the macrolide compounds have been identified, which act as antibiotics possessing a broad spectrum of activity in treating or preventing bacterial infections and protozoal infections, especially those associated with Gram-positive and Gram-negative bacteria and in particular, Gram-positive bacteria.

In one embodiment, the invention encompasses novel crystalline and amorphous forms of the macrolide of Formula I:

Formula I



In another embodiment, the invention encompasses a mixture of compounds with varying amounts of the Compound of Formula I, which forms have the requisite stability for use in preparing pharmaceutical compositions.

In another embodiment, the invention encompasses a polymorph obtained from a mixture of tiacumicins and a Compound of Formula I.

In still another embodiment, the invention encompasses novel crystalline and amorphous forms of the Compound of Formula I.

In another embodiment, the invention encompasses a pharmaceutical composition comprising a Compound of Formula I.

In another embodiment, the invention encompasses a pharmaceutical composition comprising a Compound of Formula I, wherein the Compound of Formula I is present in an amount greater than 90% by weight.

In another embodiment, the invention encompasses a pharmaceutical composition comprising one or more novel crystalline and amorphous forms of a Compound of Formula I.

In another embodiment, the invention encompasses a pharmaceutical composition comprising a mixture of tiacumicins and Compound of Formula I.

In another embodiment, the invention encompasses a pharmaceutical composition comprising a mixture of tiacumicins and at least about 75% or more by weight of Compound of Formula I. In another embodiment, the invention encompasses a pharmaceutical composition comprising a mixture of tiacumicins and at least about 80% or more by weight of Compound of Formula I. In another embodiment, the invention encompasses a pharmaceutical composition comprising a mixture of tiacumicins and at least about 85% or more by weight of Compound of Formula I. In another embodiment, the invention encompasses a pharmaceutical composition comprising a mixture of tiacumicins and at least about 90% or more by weight of Compound of Formula I. In another embodiment, the invention encompasses a pharmaceutical composition comprising a mixture of tiacumicins and at least about 95% or more by weight of Compound of Formula I. In another embodiment, the invention encompasses a pharmaceutical composition comprising a mixture of tiacumicins and at least about 99% or more by weight of Compound of Formula I.

The invention also encompasses methods for treating or preventing a disease or disorder including, but not limited to, bacterial infections and protozoal infections comprising administering to a subject, preferably a mammal, in need thereof a therapeutically or prophylactically effective amount of a composition or formulation comprising a compound of the invention.

In one illustrative embodiment, the composition or formulation comprises a mixture of compounds with varying amounts of the Compound of Formula I. In another embodiment, the composition or formulation comprises a mixture of tiacumicins and a Compound of Formula I. In still another embodiment, the composition or formulation comprises novel crystalline and amorphous forms of the Compound of Formula I. In still another embodiment, the composition or formulation comprises novel crystalline and amorphous forms of the Compound of Formula I and a mixture of tiacumicins.

In another particular embodiment, the disease or disorder to be treated or prevented are caused by toxin producing strains of *C. difficile, Staphylococcus aureus* ("*S. aureus*") including methicillin-resistant *Staphylococcus aureus* ("MRSA") and *C. perfringens*. In another particular embodiment, the disease or disorder to be treated or prevented is antibiotic-associated diarrhea.

5. BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** shows the X-ray powder diffraction patterns of a first polymorph Compound of Formula I produced from methanol and water.

FIG. **2** shows the X-ray powder diffraction patterns of a second polymorph Compound of Formula I produced from ethyl acetate.

FIG. **3** shows the effect of temperature on a mixture of tiacumicins produced from methanol and water. The DSC indicates an endothermic curve beginning at 169° C., and weight loss beginning at 223° C. The endothermic curve at about 177° C. corresponds to the melting of a first polymorph of a Compound of Formula I.

6. DETAILED DESCRIPTION OF THE DRAWINGS

6.1. General Description

The invention broadly encompasses mixtures of compounds with varying amounts of the Compound of Formula I. The inventors have surprisingly determined that the forma-

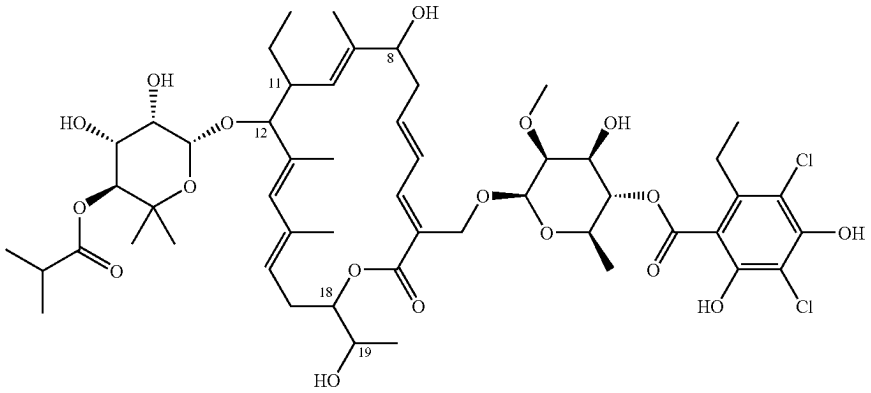
tion of crystalline polymorphic forms and amorphous forms of a Compound of Formula I and optionally mixtures of tiacumicin depends on the selection of the crystallization solvent and on the method and conditions of crystallization or precipitation.

In one embodiment the invention encompasses a mixture of tiacumicins and a Compound of Formula I. In another embodiment, the invention encompasses novel crystalline and amorphous forms of the Compound of Formula I and optionally a mixture of tiacumicins. In still another embodiment, the invention encompasses novel crystalline and amorphous forms of the Compound of Formula I and a mixture of tiacumicins. In another embodiment, the invention encompasses a mixture of comprising a first polymorph of a Compound of Formula I, a second polymorph of a Compound of Formula I, and other polymorphic forms, amorphous forms and mixtures thereof.

In another particular embodiment, the crystalline polymorphs and amorphous forms are obtained from a mixture of tiacumicins.

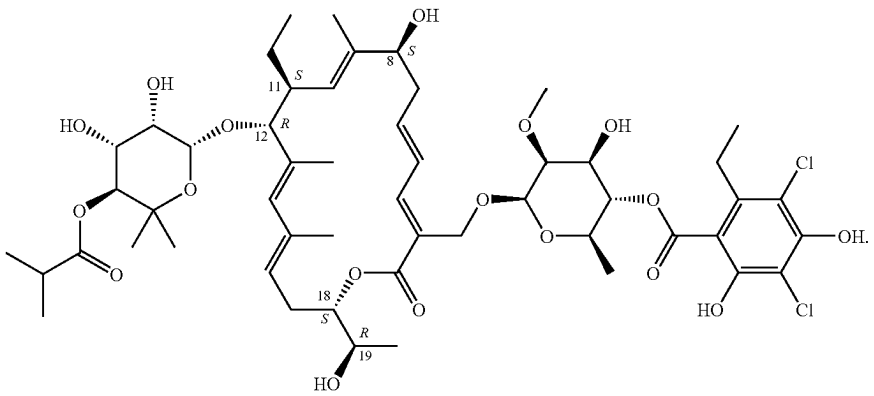
In another embodiment, a crystalline polymorph of a Compound of Formula I exhibits a representative powder diffraction pattern comprising at least peaks at the following diffraction angles 2θ of 7.7°, 15.0°, and 18.8°±0.04, preferably ±0.1, more preferably ±0.15, even more preferably ±0.2. In another embodiment, a crystalline polymorph of a Compound of Formula I exhibits a representative powder diffraction pattern comprising at least peaks at the following diffraction angles 2θ of 7.8°, 15.1°, and 18.8°±0.04, preferably ±0.1, more preferably ±0.15, even more preferably ±0.2.

In another embodiment, the polymorph has the chemical structure:



In another embodiment, the polymorph has the chemical structure of a Compound of Formula I:

Formula I



In another embodiment, the polymorph further comprises at least one compound selected from a mixture of tiacumicins.

In another embodiment, the polymorph of Formula I is present in an amount from at least about 75% to about 99.99%.

In another embodiment, the polymorph of Formula I is present in an amount of at least about 75%.

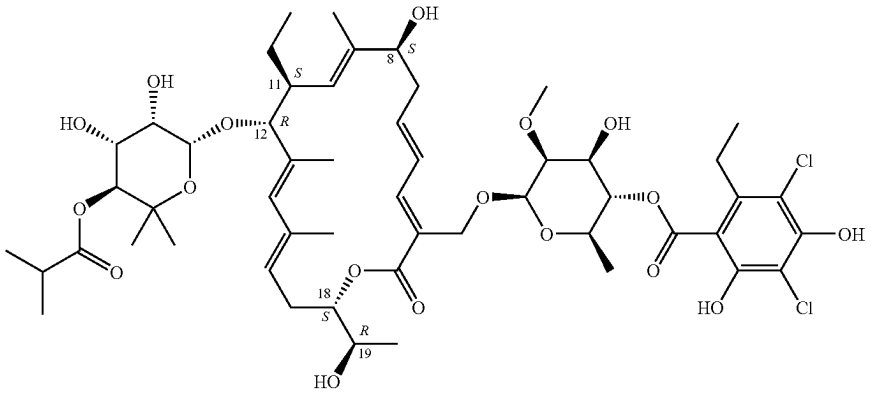
In another embodiment, the polymorph of Formula I is present in an amount of at least about 80%.

In another embodiment, the polymorph of Formula I is present in an amount of at least about 85%.

In another embodiment, the polymorph of Formula I is present in an amount of at least about 90%.

In another embodiment, the polymorph of Formula I is present in an amount of at least about 93%.

In another embodiment, the polymorph of Formula I is present in an amount of at least about 95%.



In another embodiment, the polymorph of Formula is present in an amount of at least about 99%.

In another embodiment, the crystalline polymorph is obtained from a mixture of tiacumicins that exhibits a melting point of about 163° C. to about 169° C. In another embodiment, the crystalline polymorph is obtained from a mixture of tiacumicins that exhibits a melting point of about 160° C. to about 170° C. In another embodiment, the crystalline polymorph is obtained from a mixture of tiacumicins that exhibits a melting point of about 155° C. to about 175° C.

In another embodiment, the crystalline polymorph is obtained from a mixture of tiacumicins and exhibits a DSC endotherm in the range of about 174° C. to about 186° C.; preferably 175-185° C.

In another embodiment, the crystalline polymorph is obtained from a mixture of tiacumicins that exhibits a powder diffraction pattern comprising at least peaks at the following diffraction angles 2θ of 7.7°, 15.0°, and 18.8°±0.04, preferably ±0.1, more preferably ±0.15, even more preferably ±0.2 and exhibits a melting point of about 163° C. to about 169° C.

In another embodiment, the crystalline polymorph is obtained from a mixture of tiacumicins that exhibits a powder diffraction pattern comprising at least peaks at the following diffraction angles 2θ of 7.7°, 15.0°, and 18.8°±0.04, preferably ±0.1, more preferably ±0.15, even more preferably ±0.2 and exhibits a melting point of about 160° C. to about 170° C.

Another embodiment encompasses a crystalline polymorph obtained from a mixture of tiacumicins that exhibits a powder diffraction pattern comprising at least peaks at the following diffraction angles 2θ of 7.7°, 15.0°, and 18.8°±0.04, preferably ±0.1, more preferably ±0.15, even more preferably ±0.2. In a particular embodiment, the polymorph has the chemical structure of a Compound of Formula I. In another embodiment, the crystalline polymorph further comprises at least one compound selected from a mixture of tiacumicins.

In another embodiment, a crystalline polymorph is obtained from a mixture of tiacumicins that exhibits a melting point of about 150° C. to about 156° C.

In another embodiment, a crystalline polymorph is obtained from a mixture of tiacumicins that exhibits a powder diffraction pattern comprising at least peaks at the following diffraction angles 2θ of 7.4°, 15.5°, and 18.8°±0.2 and exhibits a melting point of about 150° C. to about 156° C.

Another embodiment of the invention encompasses pharmaceutical compositions comprising a therapeutically or prophylactically effective amount of a crystalline polymorph of a Compound of Formula:

and a pharmaceutically acceptable carrier.

In a particular embodiment, the pharmaceutical composition comprises a first polymorph of a Compound of Formula I, a second polymorph of a Compound of Formula I, other polymorphic forms of a Compound of Formula I, amorphous forms of a Compound of Formula I, and mixtures thereof.

In another embodiment, the crystalline polymorph of the pharmaceutical composition has peaks at the following diffraction angles 2θ of 7.7°, 15.0°, and 18.8°±0.04, preferably ±0.1, more preferably ±0.15, even more preferably ±0.2.

In another embodiment, the crystalline polymorph of the pharmaceutical composition further comprises at least one compound selected from a mixture of tiacumicins.

In another embodiment, the Compound of Formula I is present from at least about 75% to about 99.99%, preferably about 75%, about 85%, about 95%, or about 99%.

In another embodiment, the crystalline polymorph of the pharmaceutical composition exhibits a melting point of about 163° C. to about 169° C.

Another embodiment encompasses a pharmaceutical composition comprising a crystalline polymorph of tiacumicin comprising peaks at the following diffraction angles 2θ of 7.6°, 15.4°, and 18.8°±0.04, preferably ±0.1, more preferably ±0.15, even more preferably ±0.2. In a particular embodiment, the pharmaceutical composition further comprises at least one compound selected from a mixture of tiacumicins. In another particular embodiment, the Compound of Formula I is present from about 75% to about 99.99%, preferably 75%, 85%, 95%, or 99%.

In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure

R-Tiacumicin and less than 15% of a mixture of tiacumicins. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 10% of a mixture of tiacumicins. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 7% of a mixture of tiacumicins. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 5% of a mixture of tiacumicins. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 1% of a mixture of tiacumicins. In another embodiment; the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 15% of a mixture of S-Tiacumicin. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 10% of a mixture of S-Tiacumicin. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 7% of a mixture of S-Tiacumicin. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 5% of a mixture of S-Tiacumicin. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 1% of a mixture of S-Tiacumicin. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 15% of a mixture of Lipiarmycin A4. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 10% of a mixture of Lipiarmycin A4. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 7% of a mixture of Lipiarmycin A4. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 5% of a mixture of Lipiarmycin A4. In another embodiment, the invention encompasses a pharmaceutical composition containing stereomerically pure R-Tiacumicin and less than 1% of a mixture of Lipiarmycin A4.

In another embodiment, the crystalline polymorph of the pharmaceutical composition exhibits a melting point of about 153° C. to about 156° C.

In another embodiment, the therapeutically or prophylactically effective amount is from about 0.01 mg/kg to about 1000 mg/kg, preferably 0.01, 0.1, 1, 2.5, 5, 10, 20, 50, 100, 250, or 500 mg/kg.

In another embodiment, the crystalline polymorph of the pharmaceutical composition is suitable for parenteral administration, preferably intravenous, intramuscular, or intraarterial.

In another embodiment, the crystalline polymorph of the pharmaceutical composition is suitable for peroral administration.

Another embodiment of the invention encompasses a method for treating a bacterial infection comprising administering a pharmaceutical composition comprising a polymorph of the invention to a subject in need thereof.

In a particular embodiment, the bacterial infection is in the gastrointestinal tract, particularly AAC or AAD.

6.2. Definitions

The term "antibiotic-associated condition" refers to a condition resulting when antibiotic therapy disturbs the balance of the microbial flora of the gut, allowing pathogenic organisms such as enterotoxin producing strains of *C. difficile, S. aureus* and *C. perfringens* to flourish. These organisms can cause diarrhea, pseudomembranous colitis, and colitis and are manifested by diarrhea, urgency, abdominal cramps, tenesmus, and fever among other symptoms. Diarrhea, when severe, causes dehydration and the medical complications associated with dehydration.

The term "asymmetrically substituted" refers to a molecular structure in which an atom having four tetrahedral valences is attached to four different atoms or groups. The commonest cases involve the carbon atom. In such cases, two optical isomers (D- and L-enantiomers or R- and S-enantiomers) per carbon atom result which are nonsuperposable mirror images of each other. Many compounds have more than one asymmetric carbon. This results in the possibility of many optical isomers, the number being determined by the formula 2n, where n is the number of asymmetric carbons.

The term "broth" as used herein refers to the fluid culture medium as obtained during or after fermentation. Broth comprises a mixture of water, the desired antibiotic(s), unused nutrients, living or dead organisms, metabolic products, and the adsorbent with or without adsorbed product.

As used herein and unless otherwise indicated, the terms "bacterial infection(s)" and "protozoal infection(s)" are used interchangeably and include bacterial infections and protozoal infections that occur in mammals, fish and birds as well as disorders related to bacterial infections and protozoal infections that may be treated or prevented by antibiotics such as the Compounds of the Invention. Such bacterial infections and protozoal infections, and disorders related to such infections, include the following: disorders associated with the use of antibiotics, chemotherapies, or antiviral therapies, including, but not limited to, colitis, for example, pseudo-membranous colitis, antibiotic associated diarrhea, and infections due to *Clostridium difficile, Clostridium perfringens, Staphylococcus* species, methicillin-resistant *Staphylococcus*, or *Enterococcus* including Vancomycin-resistant *enterococci*; antibiotic-associated diarrhea including those caused by toxin producing strains of *C. difficile, S. aureus* including methicillin-resistant *Staphylococcus aureus*, and *C. perfringens*; and antibiotic-associated colitis; pneumonia, otitis media, sinusitis, bronchitis, tonsillitis and mastoiditis related to infection by *Staphylococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Staphlococcus aureus*, or *Peptostreptococcus* spp.; pharynigis, rheumatic fever and glomerulonephritis related to infection by *Streptococcus pyogenes*, Groups C and G streptococci, *Clostridium diptheriae*, or *Actinobacillus haemolyticum*; respiratory tract infections related to infection by *Mycoplasma pneumoniae, Legionella pneumophila, Streptococcus pneumoniae, Haemophilus influenzae*, or *Chlamydia pneumoniae*; uncomplicated skin and soft tissue infections, abscesses and osteomyelitis, and puerperal fever related to infection by Staphlococcus aureus, coagulase-positive staphlococci (e.g., *S. epidermis* and *S. hemolyticus*), *Staphylococcus pyogenes, Streptococcus agalactiae*, Streptococcal groups C-F (minute-colony streptococci), viridans streptococci, *Corynebacterium minutissimum, Clostridium* spp., or *Bartonella henselae*; uncomplicated acute urinary tract infections related to infection by *Staphylococcus saprophyticus* or *Enterococcus* spp.; urethritis and cervicitis; and sexually transmitted diseases related to infection by *Chlamydia trachomatis, Haemophilus ducreyi, Treponema pallidum, Ureaplasma urealyticum*, or *Neiserria gonorrhea*; toxin diseases related to infection by *S. aureus* (food poisoning and Toxic Shock Syndrome), or Groups A, B and C streptococci; ulcers related to infection by

Helicobacter pylori, systemic febrile syndromes related to infection by *Borrelia recurrentis*; Lyme disease related to infection by *Borrelia burgdorferi*, conjunctivitis, keratitis, and dacrocystitis related to infection by *Chlamydia trachomatis, Neisseria gonorrhoeae, S. aureus, S. pneumoniae, S. pyogenes, H. influenzae*, or *Listeria* spp.; disseminated *Mycobacterium avium* complex (MAC) disease related to infection by *Mycobacterium avium*, or *Mycobacterium intracellulare*; gastroenteritis related to infection by *Campylobacter jejuni*, intestinal protozoa related to infection by *Cryptosporidium* spp.; odontogenic infection related to infection by viridans streptococci; persistent cough related to infection by *Bordetella pertussis*; gas gangrene related to infection by *Clostridium perfringens* or *Bacteroides* spp.; and atherosclerosis related to infection by *Helicobacter pylori* or *Chlamydia pneumoniae*. Bacterial infections and protozoal infections and disorders related to such infections that may be treated or prevented in animals include the following: bovine respiratory disease related to infection by *P. haem., P. multocida, Mycoplasma bovis*, or *Bordetella* spp.; cow enteric disease related to infection by *E. coli* or protozoa (e.g., coccidia, cryptosporidia, etc.); dairy cow mastitis related to infection by *Staph. aureus, Strep. uberis, Strep. agalactiae, Strep. dysgalactiae, Klebsiella* spp., *Corynebacterium*, or *Enterococcus* spp.; swine respiratory disease related to infection by *A. pleuro, P. multocida* or *Mycoplasma* spp.; swine enteric disease related to infection by *E. coli Lawsonia intracellularis, Salmonella*, or *Serpulina hyodysinteriae*; cow footrot related to infection by *Fusobacterium* spp.; cow metritis related to infection by *E. coli*; cow hairy warts related to infection by *Fusobacterium necrophorum* or *Bacteroides nodosus*; cow pink-eye related to infection by *Moraxella bovis*; cow premature abortion related to infection by protozoa (e.g., neosporium) urinary tract infection in dogs and cats related to infection by *E. coli*; skin and soft tissue infections in dogs and cats related to infection by *Staph. epidermidis, Staph. intermedius*, coagulase neg. *Staph*. or *P. multocida*; and dental or mouth infections in dogs and cats related to infection by *Alcaligenes* spp., *Bacteroides* spp., *Clostridium* spp., *Enterobacter* spp., *Eubacterium, Peptostreptococcus, Porphyromonas*, or *Prevotella*. Other bacterial infections and protozoal infections and disorders related to such infections that may be treated or prevented in accord with the methods of the invention are referred to in Sanford, J. P., et al., "The Sanford Guide To Antimicrobial Therapy," 27th Edition (Antimicrobial Therapy, Inc., 1996).

As used herein and unless otherwise indicated, the term "binders" refers to agents used to impart cohesive qualities to the powdered material. Binders, or "granulators" as they are sometimes known, impart cohesiveness to the tablet formulation, which insures the tablet remaining intact after compression, as well as improving the free-flowing qualities by the formulation of granules of desired hardness and size. Materials commonly used as binders include starch; gelatin; sugars, such as sucrose, glucose, dextrose, molasses, and lactose; natural and synthetic gums, such as acacia, sodium alginate, extract of Irish moss, panwar gum, ghatti gum, mucilage of isapol husks, carboxymethylcellulose, methylcellulose, polyvinylpyrrolidone, Veegum, microcrystalline cellulose, microcrystalline dextrose, amylose, and larch arabogalactan, and the like.

As used herein and unless otherwise indicated, the terms "biohydrolyzable amide," "biohydrolyzable ester," "biohydrolyzable carbamate," "biohydrolyzable carbonate," "biohydrolyzable ureide," "biohydrolyzable phosphate" mean an amide, ester, carbamate, carbonate, ureide, or phosphate, respectively, of a compound that either: 1) does not interfere with the biological activity of the compound but can confer upon that compound advantageous properties in vivo, such as uptake, duration of action, or onset of action; or 2) is biologically inactive but is converted in vivo to the biologically active compound. Examples of biohydrolyzable esters include, but are not limited to, lower alkyl esters, lower acyloxyalkyl esters (such as acetoxylmethyl, acetoxyethyl, aminocarbonyloxy-methyl, pivaloyloxymethyl, and pivaloyloxyethyl esters), lactonyl esters (such as phthalidyl and thiophthalidyl esters), lower alkoxyacyloxyalkyl esters (such as methoxycarbonyloxy-methyl, ethoxycarbonyloxyethyl and isopropoxycarbonyloxyethyl esters), alkoxyalkyl esters, choline esters, and acylamino alkyl esters (such as acetamidomethyl esters). Examples of biohydrolyzable amides include, but are not limited to, lower alkyl amides, a amino acid amides, alkoxyacyl amides, and alkylaminoalkyl-carbonyl amides. Examples of biohydrolyzable carbamates include, but are not limited to, lower alkylamines, substituted ethylenediamines, aminoacids, hydroxyalkylamines, heterocyclic and heteroaromatic amines, and polyether amines.

As used herein and unless otherwise indicated, the term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which a composition is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like.

As used herein and unless otherwise indicated, the term "Compounds of the Invention" means, collectively, a Compound of Formula I and/or pharmaceutically acceptable salts and polymorphs thereof. The compounds of the invention are identified herein by their chemical structure and/or chemical name. Where a compound is referred to by both a chemical structure and a chemical name, and that chemical structure and chemical name conflict, the chemical structure is determinative of the compound's identity. The compounds of the invention may contain one or more chiral centers and/or double bonds and, therefore, exist as stereoisomers, such as double-bond isomers (i.e., geometric isomers), enantiomers, or diastereomers. According to the invention, the chemical structures depicted herein, and therefore the compounds of the invention, encompass all of the corresponding compound's enantiomers and stereoisomers, that is, both the stereomerically pure form (e.g., geometrically pure, enantiomerically pure, or diastereomerically pure) and enantiomeric and stereoisomeric mixtures. Enantiomeric and stereoisomeric mixtures can be resolved into their component enantiomers or stereoisomers by well known methods, such as chiral-phase gas chromatography, chiral-phase high performance liquid chromatography, crystallizing the compound as a chiral salt complex, or crystallizing the compound in a chiral solvent. Enantiomers and stereoisomers can also be obtained from stereomerically- or enantiomerically-pure intermediates, reagents, and catalysts by well known asymmetric synthetic methods. The Compounds of the Invention are preferably substantially stereomerically pure. In a particular embodiment, the term "Compounds of the Invention" refers to a Compound of Formula that is greater than 75% pure, preferably greater than 85% pure, more preferably greater than 95% pure and most preferably greater than 99% pure and polymorphic form (e.g., a polymorph of Compound of Formula I) and amorphous forms thereof.

As used herein and unless otherwise indicated, "diluents" are inert substances added to increase the bulk of the formulation to make the tablet a practical size for compression. Commonly used diluents include calcium phosphate, calcium

sulfate, lactose, kaolin, mannitol, sodium chloride, dry starch, powdered sugar, silica, and the like.

As used herein and unless otherwise indicated, "disintegrators" or "disintegrants" are substances that facilitate the breakup or disintegration of tablets after administration. Materials serving as disintegrants have been chemically classified as starches, clays, celluloses, algins, or gums. Other disintegrators include Veegum HV, methylcellulose, agar, bentonite, cellulose and wood products, natural sponge, cation-exchange resins, alginic acid, guar gum, citrus pulp, cross-linked polyvinylpyrrolidone, carboxymethylcellulose, and the like.

When administered to a subject (e.g., to an animal for veterinary use or to a human for clinical use) the compounds of the invention are administered in isolated form. As used herein and unless otherwise indicated, "isolated" means that the compounds of the invention are separated from other components of either (a) a natural source, such as a plant or cell, preferably bacterial culture, or (b) a synthetic organic chemical reaction mixture, preferably, via conventional techniques, the compounds of the invention are purified. As used herein, "purified" means that when isolated, the isolate contains at least about 70% preferably at least about 80%, more preferably at least about 90%, even more preferably at least about 95%, and most preferably at least about 99% of a compound of the invention by weight of the isolate.

The term "macrolide" or "macrocycle" refers to organic molecules with large ring structures usually containing over 10 atoms.

The term "18-membered macrocycles" refers to organic molecules with ring structures containing 18 atoms.

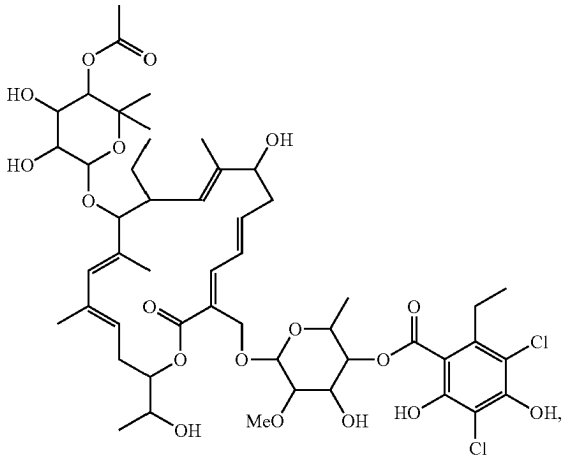
The term "MIC" or "minimum inhibitory concentration" refers to the lowest concentration of an antibiotic that is needed to inhibit growth of a bacterial isolate in vitro. A common method for determining the MIC of an antibiotic is to prepare several tubes containing serial dilutions of the antibiotic, that are then inoculated with the bacterial isolate of interest. The MIC of an antibiotic can be determined from the tube with the lowest concentration that shows no turbidity (no growth).

The term "MIC50" refers to the lowest concentration of antibiotic required to inhibit the growth of 50% of the bacterial strains tested within a given bacterial species.

The term "MIC90" refers to the lowest concentration of antibiotic required to inhibit the growth of 90% of the bacterial strains tested within a given bacterial species.

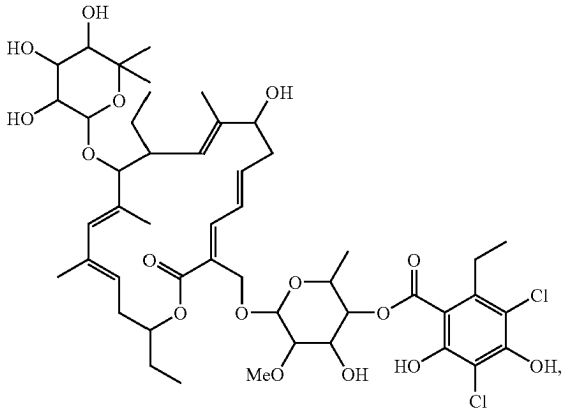
As used herein and unless otherwise indicated, the term "mixture of tiacumicins" refers to a composition containing at least one macrolide compound from the family of compounds known tiacumicins. In another embodiment, the term "mixture of tiacumicins" includes a mixture containing at least one member of the compounds known tiacumicins and a Compound of Formula I, wherein the Compound of Formula I is present in an amount of about 50%, 60%, 70%, 80%, 90%, 95%, 99%, 99.9%, or 99.99% by weight. In particular, the term "mixture of tiacumicins" refers to a compositions comprising a Compound of Formula I, wherein the Compound of Formula I has a relative retention time ("RTT") ratio of 1.0, and further comprising at least one of the following compounds:

Compound 101



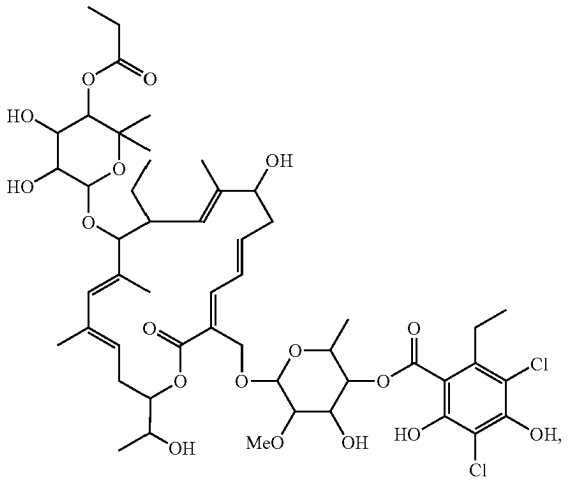
RRT ratio 0.71

Compound 102



RRT ratio 0.81

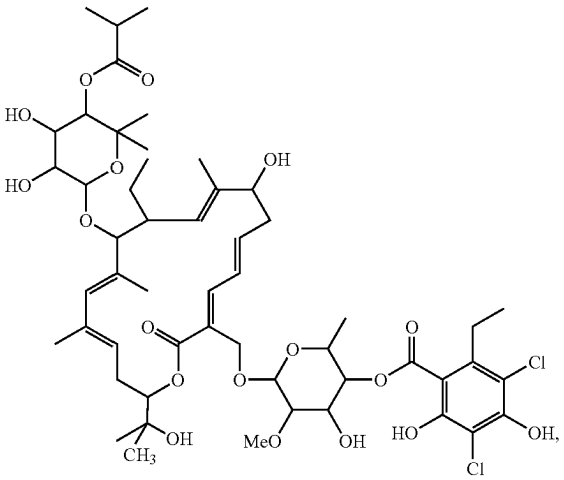
Compound 103



RRT ratio 0.84

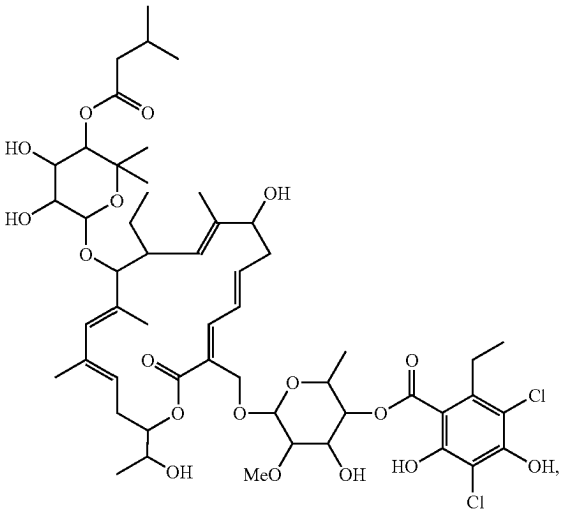
-continued

Compound 104



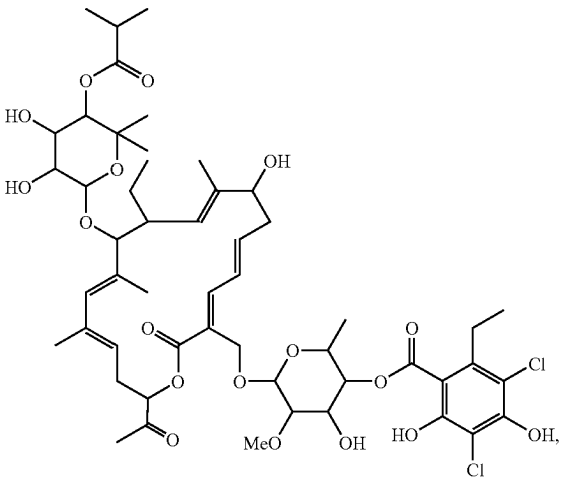
RRT ratio 1.13

Compound 105



RRT ratio 1.19

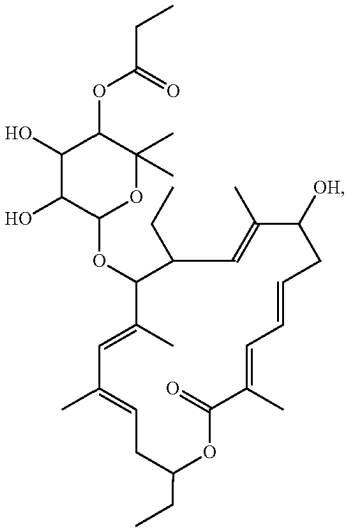
Compound 106



RRT ratio 1.24

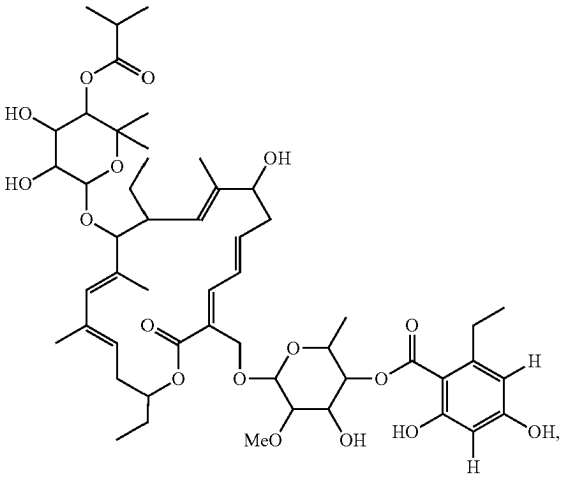
-continued

Compound 107



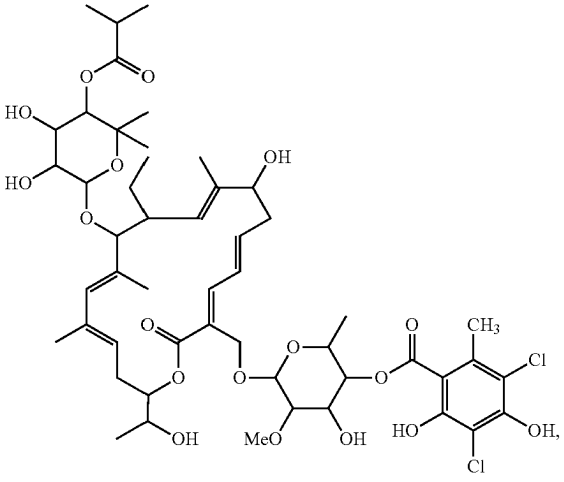
RRT ratio 1.39

Compound 108



RRT ratio 1.48

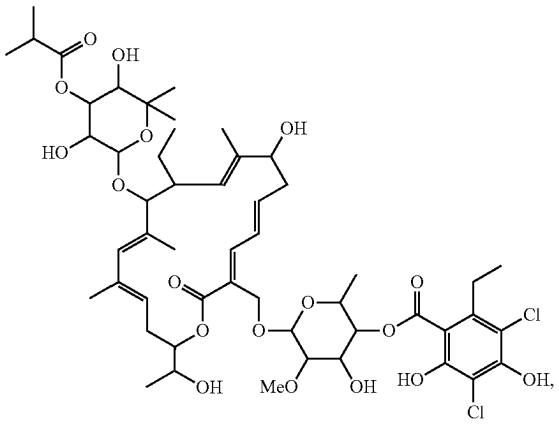
Compound 109



RTT ratio 0.89

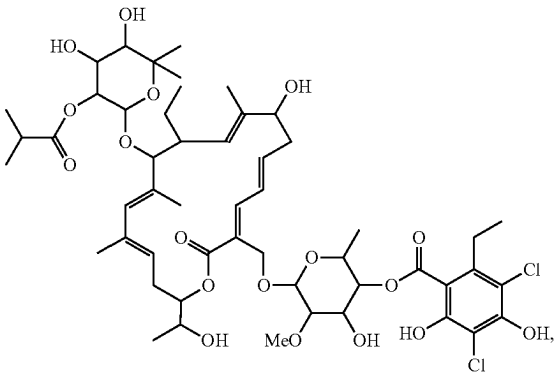
-continued

Compound 110



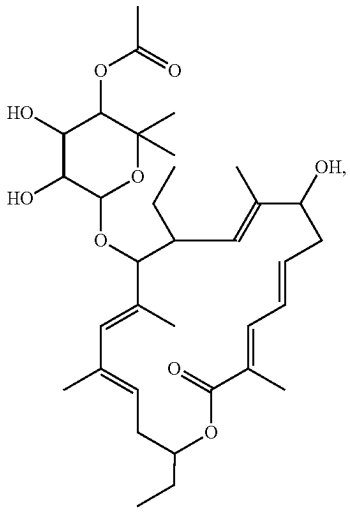
RTT ratio 0.92

Compound 111



RTT ratio 0.95

Compound 112



RTT ratio 1.10

In certain illustrative embodiments, when compound 109 is present in the mixture optionally one of compounds 110, 111, and/or 112 is also present in the mixture. Compound 109 is also sometimes referred to as Lipiarmycin A4. Compound 110 is also sometimes referred to as Tiacumicin F. Compound 111 is also sometimes referred to as Tiacumicin C. Compound 112 is also sometimes referred to as Tiacumicin A.

As used herein, and unless otherwise indicated, the terms "optically pure," "stereomerically pure," and "substantially stereomerically pure" are used interchangeably and mean one stereoisomer of a compound or a composition that comprises one stereoisomer of a compound and is substantially free of other stereoisomer(s) of that compound. For example, a stereomerically pure compound or composition of a compound having one chiral center will be substantially free of the opposite enantiomer of the compound. A stereomerically pure compound or composition of a compound having two chiral centers will be substantially free of other diastereomers of the compound. A typical stereomerically pure compound comprises greater than about 80% by weight of one stereoisomer of the compound and less than about 20% by weight of other stereoisomers of the compound, more preferably greater than about 90% by weight of one stereoisomer of the compound and less than about 10% by weight of the other stereoisomers of the compound, even more preferably greater than about 95% by weight of one stereoisomer of the compound and less than about 5% by weight of the other stereoisomers of the compound, and most preferably greater than about 97% by weight of one stereoisomer of the compound and less than about 3% by weight of the other stereoisomers of the compound.

As used herein and unless otherwise indicated, "pharmaceutically acceptable" refers to materials and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human. Typically, as used herein, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

As used herein and unless otherwise indicated, the term "pharmaceutically acceptable hydrate" means a Compound of the Invention that further includes a stoichiometric or non-stoichiometric amount of water bound by non-covalent intermolecular forces.

As used herein and unless otherwise indicated, the term "pharmaceutically acceptable polymorph" refers to a Compound of the Invention that exists in several distinct forms (e.g., crystalline, amorphous), the invention encompasses all of these forms.

As used herein and unless otherwise indicated, the term "pharmaceutically acceptable prodrug" means a derivative of a modified polymorph of a compound of Formula I that can hydrolyze, oxidize, or otherwise react under biological conditions (in vitro or in vivo) to provide the compound. Examples of prodrugs include, but are not limited to, compounds that comprise biohydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. Other examples of prodrugs include compounds that comprise oligonucleotides, peptides, lipids, aliphatic and aromatic groups, or NO, NO_2, ONO, and ONO_2 moieties. Prodrugs can typically be prepared using well known methods, such as those described in Burger's Medicinal Chemistry and Drug Discovery, 172 178, 949 982 (Manfred E. Wolff ed., 5th ed. 1995), and Design of Prodrugs (H. Bundgaard ed., Elsevier, New York 1985).

The phrase "pharmaceutically acceptable salt(s)," as used herein includes but is not limited to salts of acidic or basic groups that may be present in compounds used in the present compositions. Compounds included in the present compositions that are basic in nature are capable of forming a wide

variety of salts with various inorganic and organic acids. The acids that may be used to prepare pharmaceutically acceptable acid addition salts of such basic compounds are those that form non-toxic acid addition salts, i.e., salts containing pharmacologically acceptable anions including, but not limited to, sulfuric, citric, maleic, acetic, oxalic, hydrochloride, hydrobromide, hydroiodide, nitrate, sulfate, bisulfate, phosphate, acid phosphate, isonicotinate, acetate, lactate, salicylate, citrate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucaronate; saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate and pamoate (i.e., 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)) salts. Compounds included in the present compositions that include an amino moiety may form pharmaceutically acceptable salts with various amino acids, in addition to the acids mentioned above. Compounds, included in the present compositions, which are acidic in nature are capable of forming base salts with various pharmacologically acceptable cations. Examples of such salts include alkali metal or alkaline earth metal salts and, particularly, calcium, magnesium, sodium lithium, zinc, potassium, and iron salts.

As used herein and unless otherwise indicated, the term "prophylactically effective" refers to an amount of a Compound or Composition of the Invention or a pharmaceutically acceptable salt, solvate, polymorph, or prodrug thereof causing a reduction of the risk of acquiring a given disease or disorder. Accordingly, the Compounds of the Invention may be used for the prevention of one disease or disorder and concurrently treating another (e.g., prevention of AAC, while treating urinary AAD). In certain embodiments, the compositions of the invention are administered to a patient, preferably a human, as a preventative measure against such diseases. As used herein, "prevention" or "preventing" refers to a reduction of the risk of acquiring a given disease or disorder.

As used herein, the term "subject" can be a mammal, preferably a human or an animal. The subject being treated is a patient in need of treatment.

As used herein and unless otherwise indicated, the phrase "therapeutically effective amount" of a Compound or Composition of the Invention or a pharmaceutically acceptable salt, solvate, polymorph, or prodrug thereof is measured by the therapeutic effectiveness of a compound of the invention, wherein at least one adverse effect of a disorder is ameliorated or alleviated. In one embodiment, the term "therapeutically effective amount" means an amount of a drug or Compound of the Invention that is sufficient to provide the desired local or systemic effect and performance at a reasonable benefit/risk ratio attending any medical treatment. In one embodiment, the phrase "therapeutically effective amount" of a composition of the invention is measured by the therapeutic effectiveness of a compound of the invention to alleviate at least one symptom associated with bacterial or protazoal infections. Surprisingly, the inventors have found that therapeutically effective amounts of the compounds of the invention are useful in treating or preventing bacterial and protazoal infections.

As used herein and unless otherwise indicated, the terms "treatment" or "treating" refer to an amelioration of a disease or disorder, or at least one discernible symptom thereof, preferably associated with a bacterial or protozoal infection. In another embodiment, "treatment" or "treating" refers to an amelioration of at least one measurable physical parameter, not necessarily discernible by the patient. In yet another embodiment, "treatment" or "treating" refers to inhibiting the progression of a disease or disorder, either physically, e.g., stabilization of a discernible symptom, physiologically, for example, stabilization of a physical parameter, or both. In yet another embodiment, "treatment" or "treating" refers to delaying the onset of a disease or disorder.

6.3. Compositions of the Invention for Therapeutic/Prophylactic Administration

The invention encompasses compositions comprising a first polymorph of a Compound of Formula I, a second polymorph of a Compound of Formula I, other polymorphic forms, amorphous form or mixtures thereof of a mixture of tiacumicins with varying amounts of the Compound of Formula I.

The invention further encompasses an antibiotic composition that is a mixture of tiacumicins for use in treating CDAD as well as, AAD and AAC. The mixture of tiacumicins contains about 76 to about 100% of a Compound of Formula I, which belongs to the tiacumicin family of 18-member macrolide.

Due to the activity of the Compounds of the Invention, the compounds are advantageously useful in veterinary and human medicine. The Compounds of the Invention are useful for the treatment or prevention of bacterial and protozoal infections. In some embodiments, the subject has an infection but does not exhibit or manifest any physiological symptoms associated with an infection.

The invention provides methods of treatment and prophylaxis by administration to a patient of a therapeutically effective amount of a composition comprising a crystalline polymorph or amorphous form of a Compound of the Invention. The patient is a mammal, including, but not limited, to an animal such a cow, horse, sheep, pig, chicken, turkey, quail, cat, dog, mouse, rat, rabbit, guinea pig, etc., and is more preferably a human.

The present compositions, which comprise one or more crystalline polymorph or amorphous form of a Compounds of the Invention or a mixture of tiacumicins may be administered by any convenient route, for example, peroral administration, parenteral administration, by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with another biologically active agent. Administration can be systemic or local. Various delivery systems are known, e.g., encapsulation in liposomes, microparticles, microcapsules, capsules, etc., and can be used to administer a compound of the invention. In certain embodiments, more than one Compound of the Invention and mixture of tiacumicins is administered to a patient. Methods of administration include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, oral, sublingual, intranasal, intracerebral, intravaginal, transdermal, rectally, by inhalation, or topically, particularly to the ears, nose, eyes, or skin. The preferred mode of administration is left to the discretion of the practitioner, and will depend in-part upon the site of the medical condition. In most instances, administration will result in the release of the crystalline polymorph or amorphous form of a Compound of the Invention into the bloodstream.

In specific embodiments, it may be desirable to administer one or more crystalline polymorph or amorphous form of a Compound of the Invention locally to the area in need of treatment. This may be achieved, for example, and not by way of limitation, by local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a

suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration can be by direct injection at the site (or former site) of an atherosclerotic plaque tissue.

Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent, or via perfusion in a fluorocarbon or synthetic pulmonary surfactant. In certain embodiments, the compounds of the invention can be formulated as a suppository, with traditional binders and vehicles such as triglycerides.

In another embodiment, the a crystalline polymorph or amorphous form of a Compound of the Invention can be delivered in a vesicle, in particular a liposome (see Langer, 1990, Science 249:1527-1533; Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid.).

In yet another embodiment, the compounds of the invention can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, 1987, *CRC Crit. Ref. Biomed. Eng.* 14:201; Buchwald et al., 1980, *Surgery* 88:507 Saudek et al., 1989, N. Engl. J. Med. 321:574). In another embodiment, polymeric materials can be used (*see Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, *J. Macromol. Sci. Rev. Macromol. Chem.* 23:61; see also Levy et al., 1985, *Science* 228:190; During et al., 1989, *Ann. Neurol.* 25:351; Howard et al., 1989, J. *Neurosurg.* 71:105). In yet another embodiment, a controlled-release system can be placed in proximity of the target of the compounds of the invention, e.g., the liver, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, supra, vol. 2, pp. 115-138 (1984)). Other controlled-release systems discussed in the review by Langer, 1990, Science 249:1527-1533) may be used.

The present compositions will contain a therapeutically effective amount of a crystalline polymorph or amorphous form of a Compound of the Invention, optionally more than one crystalline polymorph or amorphous form of a Compound of the Invention, preferably in purified form, together with a suitable amount of a pharmaceutically acceptable vehicle so as to provide the form for proper administration to the patient.

In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "vehicle" refers to a diluent, adjuvant, excipient, or carrier with which a compound of the invention is administered. Such pharmaceutical vehicles can be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The pharmaceutical vehicles can be saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea, and the like. In addition, auxiliary, stabilizing, thickening, lubricating and coloring agents may be used. When administered to a patient, the compounds of the invention and pharmaceutically acceptable vehicles are preferably sterile. Water is a preferred vehicle when the compound of the invention is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid vehicles, particularly for injectable solutions. Suitable pharmaceutical vehicles also include excipients such as starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The present compositions, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

The present compositions can take the form of solutions, suspensions, emulsion, tablets, pills, pellets, capsules, capsules containing liquids, powders, sustained-release formulations, suppositories, emulsions, aerosols, sprays, suspensions, or any other form suitable for use. In one embodiment, the pharmaceutically acceptable vehicle is a capsule (see e.g., U.S. Pat. No. 5,698,155). Other examples of suitable pharmaceutical vehicles are described in "Remington's Pharmaceutical Sciences" by A. R. Gennaro.

In a preferred embodiment, the crystalline polymorph or amorphous form of a Compound of the Invention is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, a crystalline polymorph or amorphous form of a Compound of the Invention for intravenous administration is a solution in sterile isotonic aqueous buffer. Where necessary, the compositions may also include a solubilizing agent. Compositions for intravenous administration may optionally include a local anesthetic such as lidocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the crystalline polymorph or amorphous form of a Compound of the Invention is to be administered by infusion, it can be dispensed, for example, with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the compound of the invention is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

It is preferred that the compositions of the invention be administered orally. Compositions for oral delivery may be in the form of tablets, lozenges, aqueous or oily suspensions, granules, powders, emulsions, capsules, syrups, or elixirs, for example. Orally administered compositions may contain one or more optionally agents, for example, sweetening agents such as fructose, aspartame or saccharin; flavoring agents such as peppermint, oil of wintergreen, or cherry; coloring agents; and preserving agents, to provide a pharmaceutically palatable preparation. Moreover, where in tablet or pill form, the compositions may be coated to delay disintegration and absorption in the gastrointestinal tract thereby providing a sustained action over an extended period of time. Selectively permeable membranes surrounding an osmotically active driving compound are also suitable for orally administered crystalline polymorph or amorphous form of a Compound of the Invention. In these later platforms, fluid from the environment surrounding the capsule is imbibed by the driving compound, which swells to displace the agent or agent composition through an aperture. These delivery platforms can provide an essentially zero order delivery profile as opposed to the spiked profiles of immediate release formulations. A time delay material such as glycerol monostearate or glycerol stearate may also be used. Oral compositions can include standard vehicles such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Such vehicles are preferably of pharmaceutical grade.

The amount of a crystalline polymorph or amorphous form of a Compound of the Invention that will be effective in the treatment of a particular disorder or condition disclosed herein will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, in vitro or in vivo assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the compositions will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. However, suitable dosage ranges for oral administration are generally about 0.001 milligram to 1000 milligrams of a compound of the invention per kilogram body weight. In specific preferred embodiments of the invention, the oral dose is 0.01 milligram to 500 milligrams per kilogram body weight, more preferably 0.1 milligram to 100 milligrams per kilogram body weight, more preferably 0.5 milligram to 50 milligrams per kilogram body weight, and yet more preferably 1 milligram to 10 milligrams per kilogram body weight. In a most preferred embodiment, the oral dose is 1 milligram of a crystalline polymorph or amorphous form of a Compound of the Invention per kilogram body weight. The dosage amounts described herein refer to total amounts administered; that is, if more than one compound of the invention is administered, the preferred dosages correspond to the total amount of the compounds of the invention administered. Oral compositions preferably contain 10% to 95% active ingredient by weight.

Suitable dosage ranges for intravenous (i.v.) administration are 0.001 milligram to 1000 milligrams per kilogram body weight, 0.1 milligram to 100 milligrams per kilogram body weight, and 1 milligram to 10 milligrams per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Suppositories generally contain 0.01 milligram to 50 milligrams of a compound of the invention per kilogram body weight and comprise active ingredient in the range of 0.5% to 10% by weight. Recommended dosages for intradermal, intramuscular, intraperitoneal, subcutaneous, epidural, sublingual, intracerebral, intravaginal, transdermal administration or administration by inhalation are in the range of 0.001 milligram to 1000 milligrams per kilogram of body weight. Suitable doses of the compounds of the invention for topical administration are in the range of 0.001 milligram to 1 milligram, depending on the area to which the compound is administered. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems. Such animal models and systems are well known in the art.

The invention also provides pharmaceutical packs or kits comprising one or more containers filled with one or more crystalline polymorph or amorphous form of a Compound of the Invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In a certain embodiment, the kit contains more than one crystalline polymorph or amorphous form of a Compound of the Invention.

The crystalline polymorph or amorphous form of a Compound of the Invention is preferably assayed in vitro and in vivo, for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays can be used to determine whether administration of a specific compound of the invention or a combination of compounds of the invention is preferred for lowering fatty acid synthesis. The compounds of the invention may also be demonstrated to be effective and safe using animal model systems.

Other methods will be known to the skilled artisan and are within the scope of the invention.

6.4. General Synthesis of the Compounds of the Invention

The 18-membered macrocycles and analogs thereof are produced by fermentation. Cultivation of *Dactylosporangium aurantiacum* subspecies *hamdenensis* AB 718C-41 NRRL 18085 for the production of the tiacumicins is carried out in a medium containing carbon sources, inorganic salts and other organic ingredients with one or more absorbents under proper aeration conditions and mixing in a sterile environment.

The microorganism to produce the active antibacterial agents was identified as belonging to the family Actinoplanaceae, genus *Dactylosporangium* (*J. Antibiotics,* 1987, 40: 567-574 and U.S. Pat. No. 4,918,174). It has been designated *Dactylasporangium aurantiacum* subspecies *hamdenensis* 718C-41. The subculture was obtained from the ARS Patent Collection of the Northern Regional Research Center, United States Department of Agriculture, 1815 North University Street, Peoria, Ill. 61604, U.S.A., where it was assigned accession number NRRL 18085. The characteristics of strain AB 718C-41 are given in the *Journal of Antibiotics,* 1987, 40: 567-574 and U.S. Pat. No. 4,918,174.

This invention encompasses the composition of novel antibiotic agents, Tiacumicins, by submerged aerobic fermentation of the microorganism *Dactylosporangium aurantiacum* subspecies *hamdenensis*. The production method is disclosed in WO 2004/014295 A2, which is hereby incorporated by reference.

7. EXAMPLES

7.1. Preparation of the Crude Mixtures of Tiacumicins and the Subsequent Crystallization of Certain Polymorphs of the Mixtures

In an illustrative embodiment, a mixture of tiacumicins containing the Compound of Formula I is prepared by a process comprising:

(i) culturing a microorganism in a nutrient medium to accumulate the mixture in the nutrient medium; and

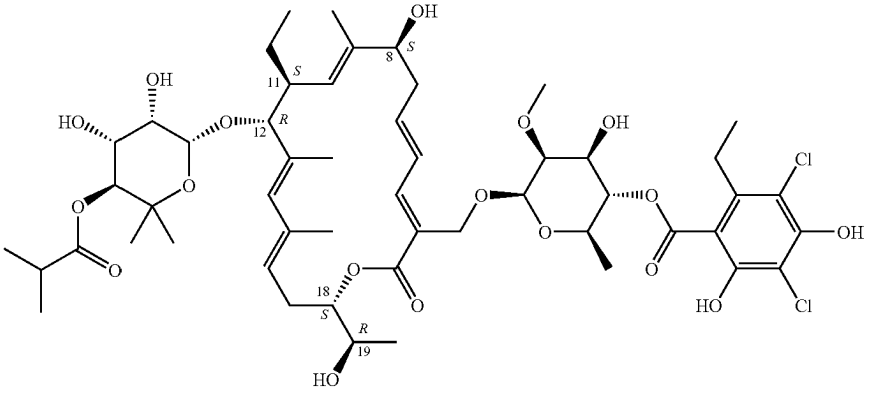
(ii) isolating the mixture from the nutrient medium; wherein the nutrient medium comprises an adsorbent to adsorb the mixture.

The nutrient medium preferably comprises from about 0.5 to about 15% of the adsorbent by weight. The absorbent is preferably an adsorbent resin. More preferably, the adsorbent resin is Amberlite®, XAD16, XAD16HP, XAD2, XAD7HP, XAD1180, XAD1600, IRC50, or Duolite® XAD761. The microorganism is preferably *Dactylosporangium aurantiacum* subspecies *hamdenensis*. The nutrient medium comprises the following combination based on weight: from about 0.2% to about 10% of glucose, from about 0.02% to about 0.5% of K_2HPO_4, from about 0.02% to about 0.5% of $MgSO_4.7H_2O$, from about 0.01% to about 0.3% of KCl, from about 0.1% to about 2% of $CaCO_3$, from about 0.05% to about 2% of casamino acid, from about 0.05% to about 2% of yeast extract, and from about 0.5% to about 15% of XAD-16 resin. The culturing step is preferably conducted at a temperature from about 25° C. to about 35° C. and at a pH from about 6.0 to about 8.0.

Upon completion of fermentation, the solid mass (including the adsorbent resin) is separated from the broth by sieving. The solid mass is eluted with organic solvents such as, for example, ethyl acetate then concentrated under reduced pressure.

7.2. Structure of R-Tiacumicin B

The structure of the R-Tiacumicin B (the major most active component) is shown below in Formula I. The X-ray crystal structure of the R-Tiacumicin B was obtained as a colorless, parallelepiped-shaped crystal (0.08×0.14×0.22 mm) grown in aqueous methanol. This x-ray structure confirms the structure shown below. The official chemical name is 3-[[[6-Deoxy-4-O-(3,5-dichloro-2-ethyl-4,6-dihydroxybenzoyl)-2-O-methyl-β-D-mannopyranosyl]oxy]-methyl]-12(R)-[[6-deoxy-5-C-methyl-4-O-(2-methyl-1-oxopropyl)-β-D-lyxo-hexopyranosyl]oxy]-11(S)-ethyl-8(S)-hydroxy-18(S)-(1(R)-hydroxyethyl)-9,13,15-trimethyloxacyclooctadeca-3,5,9,13,15-pentaene-2-one.



7.2.1 Analytical Data of R-Tiacumicin B

The analytical data of R-Tiacumicin B (which is almost entirely (i.e., >90%) R-Tiacumicin).

mp 166-169° C. (white needle from isopropanol);

$[\alpha]_D^{20}$-6.9 (c 2.0, MeOH);

MS m/z (ESI) 1079.7 $(M+Na)^+$;

^{1}H NMR (400 MHz, CD_3OD) δ 7.21 (d, 1H), 6.59 (dd, 1H), 5.95 (ddd, 1H), 5.83 (br s, 1H), 5.57 (t, 1H), 5.13 (br d, 1H), 5.09 (t, 1H), 5.02 (d, 1H), 4.71 (m, 1H), 4.71 (br s, 1H), 4.64 (br s, 1H), 4.61 (d, 1H), 4.42 (d, 1H), 4.23 (m, 1H), 4.02 (pentet, 1H), 3.92 (dd, 1H), 3.73 (m, 2H), 3.70 (d, 1H), 3.56 (s, 3H), 3.52-3.56 (m, 2H), 2.92 (m, 2H), 2.64-2.76 (m, 3H), 2.59 (heptet, 1H), 2.49 (ddd, 1H), 2.42 (ddd, 1H), 2.01 (dq, 1H), 1.81 (s, 3H), 1.76 (s, 3H), 1.65 (s, 3H), 1.35 (d, 3H), 1.29 (m, 1H), 1.20 (t, 3H), 1.19 (d, 3H), 1.17 (d, 3H), 1.16 (d, 3H), 1.14 (s, 3H), 1.12 (s, 3H), 0.87 (t, 3H);

^{13}C NMR (100 MHz, CD_3OD) δ 178.4, 169.7, 169.1, 154.6, 153.9, 146.2, 143.7, 141.9, 137.1, 137.0, 136.4, 134.6, 128.5, 126.9, 125.6, 124.6, 114.8, 112.8, 108.8, 102.3, 97.2, 94.3, 82.5, 78.6, 76.9, 75.9, 74.5, 73.5, 73.2, 72.8, 71.6, 70.5, 68.3, 63.9; 62.2, 42.5, 37.3, 35.4, 28.7, 28.3, 26.9, 26.4, 20.3, 19.6, 19.2, 18.7, 18.2, 17.6, 15.5, 14.6, 14.0, 11.4.

7.3. Preparation of a First Polymorph of R-Tiacumicin B

Another illustrative embodiment of the invention comprises a process for producing a polymorph of a Compound of Formula I from a mixture of tiacumicins comprising the steps of:

a) dissolving a crude mixture of tiacumicins containing from about 76% to about 100% of a Compound of Formula I in a minimum amount of solution comprising methanol, water, acetonitrile, acetic acid, or isopropyl alcohol mixtures thereof;

b) allowing the solution of a) to evaporate while standing at room temperature (e.g., about 22° C.) for 3 to 7 days to precipitate a first polymorph of a Compound of Formula I; and

c) separating the polymorph from the solution by techniques known in the art.

7.3.1. Illustrative Example 1 of the Preparation of a Polymorph of R-Tiacumicin B

After the fermentation process as described for example in Section 7.1, the crude material was purified by reverse phase chromatography using a Biotage Flash 75L system containing a 1.2 kg, Biotage KP-C18-HS silica column, eluted with 70:30:1, MeOH/H_2O/AcOH. The collected fractions containing 75-80% of Compound of Formula I were combined and concentrated to, one-third of the original volume to produce a precipitate. The precipitate is filtered and washed with water. The solid was dried under high vacuum to afford an off-white powder. HPLC analysis showed the powder contains about 78% of Compound of Formula I as a major product and a mixture of tiacumicins as the minor component.

The mixture of tiacumicins containing about 78% of Compound of Formula I (i.e., 50 mg) was dissolved in 2 mL of methanol followed by addition of 1 mL of water. The solution was allowed to evaporate, while standing at room temperature for 7 days to produce a crystalline precipitate. The crystal is separated from the solution by filtration. After methanol/water recrystallization, the crystals contain about 90% of Compound of Formula I based on HPLC.

7.3.2. Illustrative Example 2 of the Preparation of a Polymorph of R-Tiacumicin

After the fermentation process as described for example in Section 7.1, the crude material was purified by reverse phase

chromatography using a Biotage Flash 150 system containing a 3.75 kg, Biotage KP-C18-HS silica column, eluted with 52:48:1, EtOH/H_2O/AcOH. The collected fractions containing about 80-88% of Compound of Formula I were combined and concentrated to one-third the original volume to produce a precipitate. The precipitate was filtered and washed with water. The solid was dried under high vacuum. HPLC analysis showed the powder contains 85.4% of Compound of Formula I as a major product and a mixture of tiacumicins as the minor component.

The mixture containing about 85% of Compound of Formula I (i.e., 1000 mg) was dissolved in 20 mL of a mixture of methanol and water at ratios 1:1 methanol water. The solution was allowed to evaporate/stand at room temperature for 3 days to produce a polymorph crystalline precipitate. The crystal was separated from the solution by filtration.

The composition obtained is a mixture containing a first polymorph of a Compound of Formula I, and at least one of the tiacumicin compounds based on HPLC analysis. The composition has a melting point of 165-169° C.

7.3.3. Illustrative Example 3 of the Preparation of a Polymorph of R-Tiacumicin

After the fermentation process as described for example in Section 7.1, the crude material was purified by reverse phase chromatography using a Biotage Flash 75L system containing a 1.2 kg, Biotage KP-C18-HS silica column, eluted with MeOH/H_2O/AcOH 67:33:4 to 70:30:1. The Collected fractions containing >90% of Compound of Formula I was combined and concentrated to one-third volume. The precipitate was filtered and washed with water. The solid was dried under high vacuum. HPLC analysis showed the powder contains 94.0% of Compound of Formula I.

The solid was tested by X-ray diffraction (XRD) and Differential Scanning calorimetry (DSC) (See FIG. **2**). The X-ray diffraction of the solid shows peaks at angles 2θ of 7.7°, 15.0°, and 18.8°±0.1 indicating the solid is the form of a first polymorph of a Compound of Formula I. The DSC plot shows an endothermic curve starting at about at 169° C. and peak at 177° C.

7.3.4. Illustrative Example 4 of the Preparation of a Polymorph of R-Tiacumicin

After the fermentation process as described for example in Section 7.1, the crude material was purified by reverse phase chromatography using a Biotage Flash 75L system containing a 1.2 kg, Biotage KP-C18-HS silica column, eluted with 52:48:1, EtOH/H_2O/AcOH. The collected fractions containing >90% of Compound of Formula I were combined, one-third volume of water was added and left at room temperature overnight. The precipitate was filtered and washed with water. The solid was dried under high vacuum. HPLC analysis showed the powder contains 94.7% of Compound of Formula I.

The powder containing 94.7% of Compound of Formula I (i.e., 98 mg) was dissolved in 3 mL of methanol and then 1 mL of water was added. The solution was allowed to evaporate and stand at room temperature for 7 days to produce a crystalline precipitate. The crystals were separated from the solution by filtration and washed with methanol/water 3:1. The crystals were analyzed by X-ray diffraction.

Composition of the precipitate is a mixture comprising a Compound of Formula I based on HPLC analysis with a melting point of 166-169° C.

7.3.5. Illustrative Example 5 of the Preparation of a Polymorph of R-Tiacumicin

After the fermentation process as described for example in Section 7.1, the mixture was purified on a column, and a 0.06 gm of a mixture of tiacumicins was dissolved in 16 mL of methanol and 4 mL of water in a 20 mL vial. The vial is covered with parafilm, and pinholes were punched through. The covered vial is placed in a desiccator and stored at room temperature for ten days. Parafilm cover is then removed, and the vial is returned to desiccator. Crystalline material is produced within three to five days after the parafilm is removed. The crystalline material is washed with a solution of methanol and water and the Compound of Formula I was isolated in 75.6%.

X-ray powder diffraction pattern of the crystalline material is shown in FIG. **3** included 2θ of 7.7°, 15.0°, and 18.0°.

7.3.6. Illustrative Example 6 of the Preparation of a Polymorph of R-Tiacumicin

Preparation of a Polymorph from Isopropanol

After the fermentation process as described for example in Section 7.1, the crude material was purified by reverse phase chromatography using a Biotage Flash 150 system containing a 3.75 kg, Biotage KP-C18-HS silica column, eluted with 52:48:1, EtOH/H_2O/AcOH. The collected fractions containing 80-88% of Compound of Formula I were combined and concentrated to one-third of the original volume to produce a precipitate. The precipitate was filtered and washed with water. The solid was dried under high vacuum. HPLC analysis showed the powder contains 85.4% of Compound of Formula I.

The powder containing 85.4% Compound of Formula I (i.e., 2000 mg) was dissolved in 900 mL of isopropanol. The solution was heated to increase solubility and then filtered to remove insoluble materials. The clear solution was allowed to evaporate/stand at room temperature for 14 days to produce a crystalline precipitate. The crystal is separated from the solution by filtration.

Composition of the precipitate is a mixture comprising Compound of Formula I and at least one of other related substances based on HPLC analysis with mp of 163-165° C.

X-ray diffraction of the precipitate shows peaks at angles 2θ of 7.6° and 15.4°.

7.3.7. Illustrative Example 7 of the Preparation of a Polymorph of R-Tiacumicin

After the fermentation process as described for example in Section 7.1, and column purification, a mixture of Compound of Formula I, >90%, 15 g) was dissolved in minimum amount of methanol (from about 20 mL to about 30 mL), the solution was triturated with isopropanol (~100 mL) to produce a polymorph. The solid is separated from the solution by filtration with melting point of 165-168° C.

The XRD diagram shows a distinct polymorph pattern comprising 2 theta values of 7.5°, 15.2°, 15.7°, 18.6° 18.7°.

7.3.8. Illustrative Example 5 of the Preparation of a Polymorph of R-Tiacumicin

Preparation of a Polymorph from Acetonitrile

The mixture of tiacumicins obtained as described above and (85.44% of Compound of Formula I, 1000 mg) was dissolved in 30 mL of acetonitrile. The solution was allowed to evaporate and stand at room temperature for 12 days to

produce a crystalline precipitate. The crystal is separated from the solution by filtration, and exhibits a melting point of 165-169° C.

The XRD diagram of this crystal shows the pattern of a polymorph comprising 2 theta values of 7.8°, 15.1°, 18.8°.

7.4. Preparation of Other Polymorphs of R-Tiacumicin

Another illustrative embodiment of the invention comprises a process for producing a polymorph of a Compound of Formula I comprising the steps of:

a) dissolving crude mixture of tiacumicins containing from about 78 to about 100% of a Compound of Formula I in a minimum amount of ethyl acetate;

b) allowing the solution to evaporate and stand at room temperature for 3 to 7 days to precipitate a polymorph; and

c) separating polymorph from the solution

7.4.1. Illustrative Example 1 of the Preparation of a Polymorph of R-Tiacumicin

Preparation of Polymorph from Ethyl Acetate

After the fermentation process as described for example in Section 7.1, the crude material was purified by reverse phase chromatography using a Biotage Flash 150 system containing a 3.75 kg, Biotage KP-C18-HS silica column, eluted with 52:48:1, EtOH/H_2O/AcOH. The collected fractions containing 70-88% of Compound of Formula I was combined and concentrated to one-third volume to produce a precipitate. The precipitate is filtered and washed with water. The solid was dried under high vacuum. HPLC analysis showed the powder contains 85.4% of Compound of Formula I.

This crude tiacumicin mixture (1000 mg) was then dissolved in 30 mL of ethyl acetate. The solution was allowed to evaporate and stand at room temperature for 12 days to produce a crystalline precipitate of Polymorph B of the Compound of Formula I. The crystals were separated from the solution by filtration. The crystals have a melting point of about 153-156° C., which confirm a different polymorphic form from the first polymorph.

7.4.2. Illustrative Example 2 of the Preparation of a Polymorph of R-Tiacumicin

Preparation of a Polymorph from Methanol and Isopropanol.

After the fermentation process as described for example in Section 7.1, six different batches of crude material of varying amounts of Compound of Formula I were combined such that the combination has an average of 91% of Compound of Formula. I. The combination was dissolved in methanol and concentrated by rotary evaporation. The concentrated solution is then mixed with isopropanol, filtered, and dried by vacuum to produce a white powder with a melting point of 156-160° C.

X-ray powder diffraction of the white powder comprises 2θ values of 7.5°, 15.4°, and 18.7°.

7.4.3. Illustrative Example 3 of the Preparation of a Polymorph of R-Tiacumicin

Preparation of Polymorph B from Chloroform

After the fermentation process as described for example in Section 7.1, a crude material of tiacumicins containing Compound of Formula I was dissolved in chloroform and concentrated by evaporation at room temperature to produce a solid with a melting point of 156-160° C.

7.4.4. Illustrative Example 4 of the Preparation of a Polymorph of R-Tiacumicin

Preparation of a Polymorphic Form from Acetone

After the fermentation process as described for example in Section 7.1, a crude material of tiacumicins containing Compound of Formula I was dissolved in acetone and concentrated by evaporation at room temperature to produce a solid with a melting point of 156-160° C.

7.5. Preparation of Amorphous Forms of Compound of Formula I

Preparation of Amorphous Mixture of Tiacumicins

The amorphous mixture of tiacumicins was obtained after column purification without any further processing steps. Alternatively, chloroform or acetone may be added to the mixture of tiacumicins and the solvent is evaporated to form the amorphous product.

X-ray powder diffraction of the product exhibits no defined diffraction peaks.

8. EXPERIMENTAL DATA

8.1. Polymorph Experimental Data

A first polymorph of a Compound of a Compound of Formula I is characterized by Differential Scanning Calorimetry ("DSC") and powder X-Ray Diffraction ("XRD").

The DSC plot of the polymorph shows an endothermic curve at 177° C.

The XRD diagram (reported in FIG. **1**) shows peaks comprising at diffraction angles 2θ of 7.7°, 15.0°, 18.8°. The XRD was analyzed with a Phillips powder Diffractometer by scanning from 20 to 70 degrees two-theta at 1.0 degree per minute using Cu K-alpha radiation, at 35 kV and 20 ma. The instrumental error (variant) is 0.04 (2 theta value).

The melting point of the mixtures containing various amounts of Compound of Formula I is summarized in Table 1. All of the products with at least 85% of a Compound of Formula I in the form of a polymorph appear to have a melting point in the range of 163-169° C. measured by Melting Point apparatus, MEL-TEMP 1001.

TABLE 1

Melting point of polymorph mixtures in different solvent conditions

No.	Compound of Formula I Content (%) of the crystalline material	Mp (° C.)	Crystallization Solvent
1	85	165-169	MeOH/Water
2	85	163-165	Isopropanol
3	85	164-168	Acetonitrile
4	90	165-168	MeOH/Isopropanol
5	94	166-169	MeOH/Water
6	95	166-169	MeOH/Water
7	98	163-164	MeOH/Isopropanol

Composition of the a polymorphic crystal from a mixture comprising Compound of Formula I and optionally at least on compound that is a mixture of tiacumicins based on HPLC analysis with a melting point of 166-169° C.

X-ray diffraction of a polymorphic crystal shows characteristic peaks at angles 2θ of 7.8°, 15.0°, 18.8°, and 23.9°. Table 2 is a listing of the obtained X-ray diffraction peaks for first polymorph of R-Tiacumicin from Experiment 7.2.2.

TABLE 2

X-ray diffraction peaks for a First Polymorph from Experiment 7.3.2.

Two-Theta	Relative Intesity
3.3568	44.0000
3.4400	47.0000
7.7815	112.0000
10.1575	32.0000
13.6023	21.0000
15.0951	139.0000
17.0178	18.0000
18.8458	36.0000
19.3771	9.0000
20.0300	16.0000
20.4842	10.0000
23.9280	136.0000
24.8338	10.0000
25.0889	19.0000
25.7256	10.0000
30.9126	75.0000
31.9970	10.0000
34.4507	30.0000

Table 3 is a listing of the obtained X-ray diffraction peaks for Polymorph from Experiment 7.3.6.

TABLE 3

X-ray diffraction peaks for a Polymorph from Experiment 7.3.6.

Two-Theta	Relative Intensity
3.2978	41.0000
7.5615	400.0000
9.9482	21.0000
15.4289	31.0000
22.0360	20.0000
22.5361	20.0000
24.9507	12.0000

TABLE 3-continued

X-ray diffraction peaks for a Polymorph from Experiment 7.3.6.

Two-Theta	Relative Intensity
29.5886	10.0000
34.8526	19.0000
37.7092	17.0000
40.4361	13.0000
42.2446	18.0000

8.2. Second Polymorph of R-Tiacumicin Experimental Data

A second polymorph of Compound of Formula I is also characterized by Differential Scanning Calorimetry (DSC) and powder X-Ray Diffraction (XRD).

The DSC plot of polymorph B shows an endothermic curve at 158° C. The XRD diagram shows peaks comprising at the values of the diffraction angles 2θ of 7.6°, 15.4° and 18.8°. Polymorph B has a melting point in the range of 153-156° C. measured by Melting Point apparatus, MEL-TEMP 1001.

It is believed that crystalline polymorphic forms of Compounds of Formula I other than the above-discussed A and B exist and are disclosed herein. These crystalline polymorphic forms, including A and B, and the amorphous form or mixtures thereof contain varying amounts of Compound of Formula I and in certain cases mixtures of tiacumicins can be advantageously used in the production of medicinal preparations having antibiotic activity.

X-ray powder diffraction of the crystals is shown in FIG. **3** with peaks at angles 2θ of 7.5°, 15.7°, and 18.9°±0.04 indicating the presence of Polymorph B.

The DSC plot of Polymorph B shows an endothermic curve starting at about at 150° C. and peak at 158° C.

Table 4 is a summary of the various data that was isolated for illustrative crystallization lots.

TABLE 4

Data Summarizing Various Lots

No.	Compound of Formula I Content (%)	Mp (° C.)	DSC (° C.) Peak	XRD (2 theta)	Crystallization Solvent
1	76.3	155-158		7.7, 15.0, 18.8,	MeOH/Water
2	85.3	159-164	180	7.8, 14.9, 18.8,	MeOH/Water
3	85.4	163-165		7.6, 15.4	Iso-propanol (IPA)
4	85.4	164-168		7.9, 15.0, 18.8	Acetonitrile
5	85.4	153-156		7.5, 15.7, 18.9	EtOAc
6	90	165-168		7.5, 15.2, 15.7, 18.6	MeOH/Isopropanol
7	97.2	160-163	177	7.4, 15.4, 18.7	IPA
8	94.0	166-169	177	7.6, 15.1, 18.6	MeOH/Water
9	97.2	167-173	187	7.8, 14.8, 18.8	MeOH/Water
10	96.7		160	7.5, 15.4, 18.8	EtOAc
11	98.3	163-164	178	7.7, 15.0, 18.8	MeOH/IPA

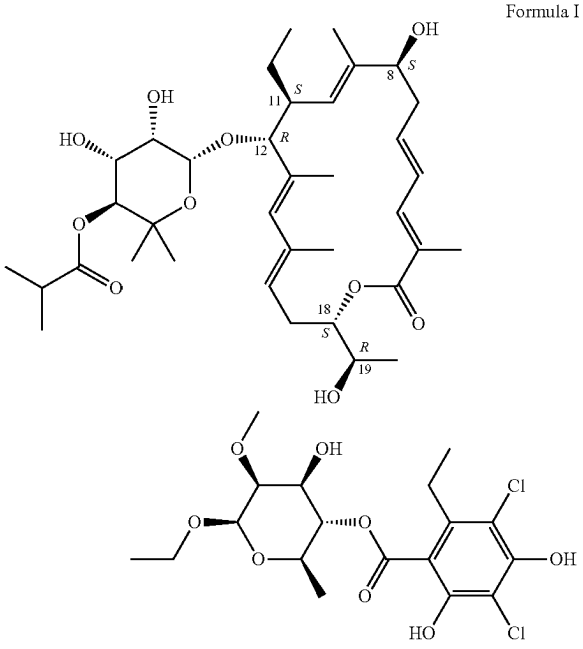
The present invention is not to be limited in scope by the specific embodiments disclosed in the examples which are intended as illustrations of a few aspects of the invention and any embodiments which are functionally equivalent are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art and are intended to fall within the appended claims.

A number of references have been cited, the entire disclosures of which are incorporated herein by reference.

What is claimed is:

1. A method of treating a bacterial infection in a subject in need thereof comprising administering to said subject a therapeutically effective amount of a composition comprising a polymorphic form of a compound of Formula I:

Formula I



wherein the polymorphic form of a compound of Formula I is characterized by a powder x-ray diffraction pattern wherein said x-ray diffraction pattern comprises peaks at diffraction angles 2θ of 7.7°, 15.0°, and 18.8°±0.2.

2. The method of claim **1**, wherein the administration is oral.

3. The method of claim **1**, wherein the amount is from about 0.001 mg to about 1000 mg.

4. The method of claim **1**, wherein the composition comprising a polymorphic form of a compound of Formula I is a solid dosage form.

5. The method of claim **1**, wherein the polymorphic form of the compound of Formula I is present in the composition with at least about 85% of the total weight of tiacumicins.

6. The method of claim **1**, wherein the polymorphic form of the compound of Formula I is present in the composition with at least about 90% of the total weight of tiacumicins.

7. The method of claim **1**, wherein the polymorphic form of the compound of Formula I is present in the composition in at least about 93% of the total weight of tiacumicins.

8. The method of claim **1**, wherein the polymorphic form of the compound of Formula I is present in the composition in at least about 95% of the total weight of tiacumicins.

9. The method of claim **1**, wherein the polymorphic form of the compound of Formula I is present in the composition in at least about 99% of the total weight of tiacumicins.

10. The method of claim **1**, wherein the bacterial infection is caused by *Clostridia*.

11. The method of claim **10**, wherein the bacterial infection is caused by *Clostridium difficile*.

12. The method of claim **1**, wherein the bacterial infection is a gastrointestinal infection.

13. The method of claim **1**, wherein the bacterial infection is *Clostridium difficile* infection.

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